The impact of respiratory disease on production in the pig industry in Great Britain

Charlotte Marie Evans BSc.

Thesis submitted in partial fulfilment for the degree of Doctor of Philosophy

Department of Biological Sciences

University of Warwick

2nd February 2010

Table of Contents

Table of Figures

Table of Tables

Acknowledgements

My most sincere gratitude goes to my two supervisors, Prof. Laura Green and Prof. Graham Medley. I am thankful to have had two excellent supervisors who have maintained my excitement in this project throughout. Thank you for being so patient and encouraging, for giving me excellent advice and support and for giving me independence to develop my own ideas and skills. Specifically, I would like to thank Graham for introducing me to infectious disease epidemiology as an undergraduate, for inspiring me then and ever since and for teaching me to always think of more than one explanation. I would like to thank Laura for having faith in me from the beginning, for fuelling my passion in animal health and for always pushing me to achieve my goals.

Thank you to Dr. James Nokes and Dr. Gerdien van Schaik for examining this thesis.

The data used in this thesis was collected during a previous DEFRA-funded study on postweaning multisystemic wasting syndrome (PMWS). Thank you to all the farmers and veterinarians who

participated in this study, and to all field technicians and research staff involved in data collection and processing: Amy Kilbride, Jane Slevin, Kerry Woodbine, Megan Turner, Emma Novell, Charlotte Boss, Fiona Boyd, Martin Crockett, Maureen Horne, Lucy Wilson and Bart ven den Borne. This thesis would not have been possible without funding from the BBSRC and from BPEX. Specifically, I would like to thank Mark Wilson and Derek Armstrong from BPEX for their commitment to the PhD.

Thank you to Simon Creasey for being so generous with his time and helping me to learn how to write code and use Matlab. Thank you also to Dr. Mike Tildesley, Prof. Graham Medley and Sam Mason for their help with writing code and helping me with using databases.

There have been many friends and colleagues who have made the last four years enjoyable. My sincere thanks go to all past and present members of the populations and disease group at Warwick for their input into the PhD and for lots of advice and suggestions. In particular, I would like to thank Megan, Claire, Amy, Jasmeet and Alicia for being such good friends and for their company, support and motivation. Specifically, thank you to Jasmeet for teaching me a lot about myself and the difference a positive attitude can make, to Amy for teaching me

about being rational, how to think over problems logically and how to deal with them and to Alicia for keeping me sane and making me laugh.

Thank you to all my friends and family who have helped me to remember that there is a life outside of my PhD. Thank you to Mum, Dad, Hannah, Dave, Tamra, Nan, Peter, Suzi, Emma-Jane and Nigel. Finally, thank you to Steve for his unselfish support throughout, for his calm and reassuring attitude, for endless discussions on research ideas and for making it possible for us to have our own pigs.

Dedication

This is for Steve, for making me believe that anything is achievable

"We learn wisdom from failure much more than from success. We often discover what will do, by finding out what will not do; and probably he who never made a mistake never made a discovery." **Samuel Smiles**

Declaration

All work presented in this thesis is the result of original research conducted by myself, except where otherwise stated in the text and in the acknowledgements. This thesis or any part of it has not been submitted or partially submitted for a degree at any other university.

The contents of Chapter 3 have been published in:

Evans, C. M., Medley, G. F. and Green, L. E (2008). Porcine reproductive and respiratory syndrome virus (PRRSV) in GB pig herds: farm characteristics associated with heterogeneity in seroprevalence. *BMC Veterinary Research.* **4,** 48.

The contents of Chapter 4 are in press:

Evans, C. M., Medley, Creasey, S. J., G. F., Green., L. E. (2009). A stochastic mathematical model of the within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV): fade-out and persistence. *Preventive Veterinary Medicine.*

Conference proceedings:

Evans, C. M., Medley, G. F., Creasey, S. J., Green., L. E. (2009). A mathematical model of the within-herd transmission dynamics of PRRSV based

on data from 103 herds. *Proceedings of the Pig Veterinary Society meeting, Bristol, UK*

Evans, C. M., Medley, G. F., Creasey, S. J., Green., L. E. (2009). Within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV) following single introduction. *Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, London, UK*

Summary

This thesis presents research on the epidemiology of respiratory diseases in pig herds in GB and their impact on pig morbidity and mortality. The role of management, characteristics of the herd, presence of multiple pathogens and control and elimination strategies were considered.

Questionnaires were completed by veterinarians that attended 116 pig herds in GB. Pathogens were clustered on individual farms, suggesting similar risk factors for infection / persistence. Management factors were investigated for their association with the within and between-herd variability in pig antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in 103 pig herds. Factors that were important included close proximity to other pig herds, having >250 sows and not isolating purchased stock or not isolating for long enough.

Considering the possible fade out of PRRSV, the within-herd transmission dynamics were investigated using a mathematical model. There was a high frequency of fade out in breeding pigs before virus reached young stock and increased persistence in young stock, in large herds, herds with increased contact between age groups and herds that had frequent re-introduction of virus. Results provided evidence for apparent erratic behaviour of PRRSV within and between herds.

Mathematical models were also used to investigate the range of impacts of PRRSV on disease in a herd and to test strategies for control and elimination. PRRSV was difficult to eliminate without targeting both rearing pigs and sows. Rapid vaccination of sows once there was an increase in preweaning still births reduced the spread of virus to rearing pigs.

Results highlighted that in areas of GB where the density of pigs is low it might be possible to control PRRSV through elimination. In larger herds in pig dense regions elimination might be difficult and control might give more stability. The long-term benefits of elimination will depend on (re)-introduction of virus from within and outside the herd but significant improvements in production might not be observed unless several respiratory pathogens are eliminated from a herd.

List of Abbreviations

DNA Deoxyribose nucleic acid

ELISA Enzyme-linked immunosorbent assay

GB Great Britain

Ig Immunoglobulin

IRPC Relative index (*100) of the civtest suis ELISA

PCR Polymerase chain reaction

PCV2 Porcine circovirus type 2

PMWS Post-weaning multisystemic wasting syndrome

PRRS Porcine reproductive and respiratory syndrome

PRRSV Porcine reproductive and respiratory syndrome virus

RNA Ribonucleic acid

US United States

1 Chapter 1: Introduction

Respiratory infections are associated with morbidity and mortality in pigs worldwide, causing reduced daily weight gain and feed conversion ratio, an overuse of antibiotics and reduced animal welfare. The subject of this thesis is respiratory infections in the pig population in GB and their impact on production. In this Chapter, the context and methods are outlined.

1.1 Pig production in Great Britain

Pig production has changed considerably in the majority of pig producing countries in the last 25 years, with herds becoming larger and situated more closely geographically according to climate and available resources. In the UK, there has been a 125% increase in the number of herds with >500 sows from 1980 – 2007. Herds are clustered, with 71.4% situated in the East of England, Yorkshire and the Humber in 2007 (Table 1.1). This increase in herd size is associated with an increase in the number of pigs and sites and increased movements on- and off-farm. In the UK, 45% of breeding sows (breeding females) are culled and replaced each year (BPEX pig yearbook, 2006). Thus for a 100 and 500 sow herd, 45 and 225 gilts (young breeding females) are purchased per year respectively. Replacement gilts can either be home bred or purchased

from other herds. The way gilts are purchased within the industry represents a pyramidal structure. This consists of a small number of nucleus herds that breed replacement stock for a larger number of multiplier herds, which, in turn, breed replacement gilts for an even larger number of commercial herds.

June 2007	SOWS IN PIG HERD SIZE GROUPS					
	0 – 50	50 - 100	100 – 150	150 - 250	250 - 500	> 500
Region						
North East	72	7	0	5	0	0
North West	257	16	9	8	0	0
Yorkshire and Humber	320	77	43	62	51	32
East Midlands	270	33	21	13	18	11
West Midlands	305	28	10	14	6	0
Eastern	323	43	27	40	31	53
London	7	0	0	0	0	0
South East	414	17	10	13	11	12
South West	811	42	15	24	13	11

June 1980	SOWS IN PIG - HERD SIZE GROUPS					
	0 - 50	50 – 100	100 - 150	150 – 250	250 - 500	> 500
Region						
North East	295	25	8	0	0	0
North West	1,046	94	41	19	8	0
Yorkshire and Humber	1,663	294	116	99	33	12
East Midlands	988	124	39	36	15	5
West Midlands	1,132	115	32	32	8	0
Eastern	1,788	439	154	88	34	11
London	28	0	0	0	0	0
South East	948	200	62	32	23	10
South East (inc. London)	976	204	62	34	23	10
South West	2,488	204	61	35	14	5

Table 1.1 Number of holdings with sows in-pig by herd size (Agricultural and horticultural survey for England in 2007 and Agricultural census for England and Wales in 1980)

1.2 The structure of a pig herd

Spatial heterogeneity exists, both within and between pig herds (Figure 1.1). Pigs are housed in pens and buildings according to their age (and therefore weight for rearing pigs). This permits more appropriate feeding and management for different groups. Sows are usually segregated according to whether they are due to be served, pregnant or lactating (with their newborn piglets) and have a 21 week cycle. Sows go through this cycle an average of 6 times before they are culled from a herd (BPEX). Each time they progress through this cycle, they age by 1 parity. Piglets remain with sows for four weeks and then join the rearing herd. The rearing herd consists of weaner, grower and finisher stages; each stage consists of pens of pigs born the same week. Pigs move through the rearing herd and are slaughtered at approximately 24 weeks of age (BPEX pig yearbook, 2006). In outdoor herds, sows farrow within farrowing arcs in individual paddocks. Piglets remain with sows until four weeks of age and then join other litters of piglets after weaning. Pens of pigs that are weaned the same week are either brought indoors or finished outdoors in outdoor pens.

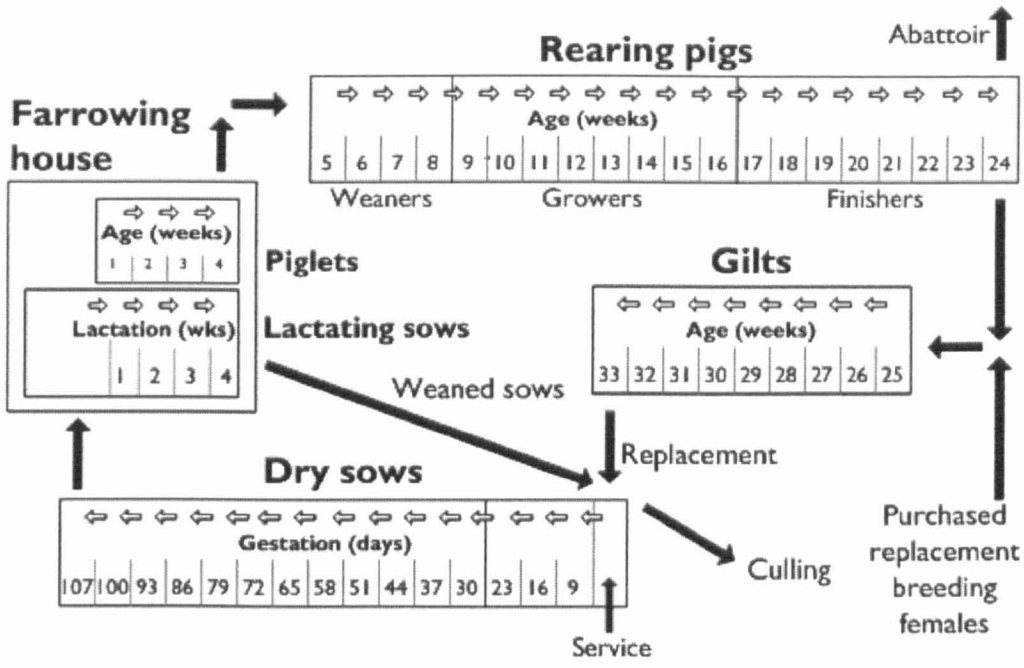


Figure 1.1 The age structure of a pig herd in GB and weekly movements of pigs

1.3 The epidemiology of infectious diseases within pig herds

Pathogens can be introduced in to a herd via purchasing infectious replacement pigs or via vectors such as birds, insects, aerosol or semen. External biosecurity can reduce the probability of introduction of a pathogen. This might include effective cleaning of vehicles that transport pigs to slaughter, preventing access of visitors that have been in contact with pigs and by isolating purchased stock with the intention that they are not infectious when they enter the herd. Once in a herd, the presence of a pathogen might be constant or it might fade out. This depends on the characteristics of the pathogen, i.e. its duration of infectiousness and ability to exist outside of the host. It will also depend on the number of susceptible pigs in

the herd and the contact structure. This determines the probability of contact between susceptible and infectious pigs. The presence of an infectious pathogen within a pig herd reduces production efficiency by causing disease and may require veterinary attention for diagnosis, treatment and control. Depending on the type of pathogen, control might require a change in management such as use of antibiotics, vaccines or change in pig flow, purchasing strategies and / or biosecurity.

The characteristics and management of the pig herd influences transmission dynamics of the pathogen and the effectiveness of different control strategies. Increases in herd size and stocking density over the last 25 years are thought to be associated with an increase in the incidence and prevalence of respiratory disease because of larger susceptible populations and increased contact between pigs. Close proximity of herds also increases the probability of between herd transmission, which has been observed for *Mycoplasma hyopneumoniae* and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (Stark *et al.*, 1998; Pitkin *et al.*, 2009). A high number of pig movements between herds was implicated in the rapid dissemination of PRRSV throughout the pig industry in 1991 / 1992 (Edwards, 1992).

1.4 Respiratory disease

Respiratory disease is most commonly observed in pigs post-weaning. Reasons for this include the moving and mixing of pigs at weaning and formation of larger group sizes and consequently increased contact between pigs, including fighting, caused by the establishment of a social hierarchy. Respiratory disease has been reported as the most important cause of morbidity and mortality in pigs post-weaning (Straw *et al.*, 1983; Losinger *et al.*, 1998). Respiratory disease has been reported to be attributable to 39.1% of all deaths in post-weaning pigs in the USA (Losinger *et al.*, 1998) and also causes reduced growth rate (Huhn, 1970; Burch, 1982; Christensen *et al*, 1995) and reduced feed conversion efficiency (Goodwin, 1971; Straw *et al.*, 1989). Zimmerman *et al.*, (1973) reported that respiratory disease reduced growth rate and feed efficiency by up to 26% and 20% respectively and Straw *et al.*, (1989) reported a reduction in mean daily weight gain of 37.4g for every 10% of a pig's lungs affected by lesions as a result of respiratory infection. Lower feed intake is thought to result from depressed appetite (Straw *et al.*, 1990). Presence of respiratory disease also has implications for animal welfare and the overuse of antibiotics. Despite a 37% decrease in the number of pigs being slaughtered in the UK from 1998-2002 and the ban on antimicrobial growth promoters, the use of antimicrobials has not decreased (Burch, 2005). It is likely that antimicrobials are currently being used as an alternative to good management practices, providing a means of allowing pigs to survive under less than favourable conditions.

1.4.1 Aetiology of respiratory disease

The porcine respiratory disease complex (PRDC) is a term used to describe respiratory disease observed in individual pigs of 14-20 weeks of age and involves multiple respiratory pathogens, including PRRSV, porcine circovirus type 2 (PCV2), porcine respiratory coronavirus, influenza virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Pasteurella multocida* and *Streptococcus suis* (Thacker, 2001). Infection with more than one of these pathogens has been associated with more severe clinical disease within individual pigs in experimental studies (Van Reeth *et al.*, 1996; Thacker *et al.*, 1999; Thacker *et al.*, 2001; Harms *et al.*, 2001; Rovira *et al.*, 2002; Opriessnig *et al.*, 2004). Many of these pathogens can act as opportunistic agents on an already compromised immune system.

1.5 Porcine reproductive and respiratory syndrome virus (PRRSV)

PRRSV was first isolated in North America in 1987 (Hill *et al.*, 1990) and in Europe in 1990 (Lindhaus and Lindhaus, 1991). In the UK, clinical signs of PRRSV infection were first observed in South Humberside in England, in May 1991 (Edwards, 1992). Its appearance also coincided with a new strain of swine influenza, H1N1, which appeared February to May 1992 (Potter, 1994).

PRRSV is an enveloped positive single stranded RNA virus (Wensvoort *et al.*, 1991). The virus belongs to the *Arteriviridae* family of viruses (genus Arterivirus, order Nidovirales) (Meulenberg *et al.*, 1993). The genome is approximately 15kb and consists of 9 open reading frames which encode structural glycoproteins, a matrix protein and a nucleocapsid protein (Meulenberg *et al.*, 1993; Meulenberg *et al.*, 1995). The virus has a high mutation rate and consequently, multiple strains have been reported. The European (Lelystad) and North American (VR-2332) strains are so genetically diverse (between 89-94% homology) (Andreyev *et al.*, 1997) that some authors have argued that they are likely to belong to different genotypes (Meng *et al.*, 1995) and evolved separately (Hanada *et al.*, 2005). However, genetic differences have not necessarily been associated with geographical distance (Pesch *et al.*, 2005) and high evolution rates of the European reference strain VR2332 have been observed within individual pigs, even as few as 60 days after challenge (Chang *et al.*, 2002).

Although there were no outbreaks of PRRS recorded before 1987 (Christianson *et al.*, 1994), antibodies were present in sera from pigs collected in 1979 (Carman *et al.*, 1995). This might suggest that PRRSV was present long before it emerged. Some authors have hypothesised that PRRSV has been present in pig herds for much longer previous to its emergence, but that the increase in herd size led to its presentation as an important disease (Zimmerman, 2003). It is possible that it evolved from another RNA virus from within the same family of viruses. These include Lactate Dehydrogenase-elevating Virus, Simian Haemorrhagic Fever Virus and Equine Arteritis Virus (Meulenberg *et al.*, 1993).

1.5.1 Transmission of PRRSV

1.5.1.1 Horizontal transmission

Pigs are the only natural host for PRRSV. Most transmission occurs horizontally, by nose-nose contact (Wills *et al.*, 1997), although virus has been detected in tonsils, lymphnodes, lungs, muscle tissue (Bloemraad *et al.*, 1994) and urine (Wills *et al.*, 1997). Virus might therefore be transmitted via contaminated needles, fighting and via close contact with slurry and / or infected carcasses. The virus does not survive very well outside the host and its half life is significantly reduced by extremes of pH, temperature (Bloemraad *et al.*, 1994) and mild disinfectants (Shirai *et al.*, 2000). This might explain why field and experimental studies have indicated a low transmissibility of PRRSV between pigs. Houben *et al.*, (1995) observed subpopulations of viraemic and susceptible pigs within single pens, even after being housed together for 12 weeks. Whilst transmission to sentinel pigs over 1 - 2.5m has been demonstrated without nose-nose contact (Wills *et al.*, 1997, Otake *et al.*, 2002a, Trincado *et al.*, 2004), transmission between buildings has only been demonstrated in some studies (Otake *et al.*, 2002a, Trincado *et al.*, 2004; Pitkin *et al.*, 2009).

Brockmeier and Lager (2002) hypothesised that PRRSV is not very transmissible because it does not cause severe coughing and therefore pigs do not expel as many virus particles into the air. Other authors have suggested that, compared with

experimental studies such as those described above, the challenge dose in the field is likely to be higher because there are more infectious pigs (Otake *et al.*, 2002a) and other infectious pathogens, which could increase coughing rates.

1.5.1.2 Vertical transmission

Transmission of PRRSV is possible from sow to piglet *in utero* (Christianson *et al.*, 1992). The probability of transmission increases with gestation, with transmission possible from 90 days gestation (Mengeling *et al.*, 1996) and conflicting reports of transmission before this time (Prieto *et al.*, 1996; Kranker, *et al.*, 1998; Mengeling *et al.*, 1994). Kranker *et al.*, (1998) inoculated sows at different stages of gestation with a European strain of the virus and reported that the percentage of piglets born alive dropped from 77.3% to 43.9% when sows were inoculated at 80-90 days, compared with 42-43 days gestation respectively.

Virus can replicate in unborn piglets (Christianson, *et al.*, 1992) and has been isolated from stillborn and live piglets at birth (Botner *et al.*, 1994, Kranker *et al.*, 1998). Houben *et al.*, (1995) reported that transmission was unlikely amongst pigs with maternal immunity. Other authors have reported that most pigs are seronegative following the waning of maternal immunity and before entering finishing accommodation (Nodelijk *et al.*, 1997), suggesting that pigs that are born viraemic contribute little or nothing to transmission of virus unless they have no maternal immunity.

1.5.1.3 Vectors

Despite evidence of low transmissibility of PRRSV, some authors have suggested farm to farm transmission without movements of infectious pigs (Edwards *et al.*, 1992, Mortensen *et al.*, 2002). It is also possible that insects and birds may harbour virus (Kristensen *et al.*, 2004, Brockmeier and Lager, 2002, Zimmermann *et al.*, 1997, Otake *et al.*, 2004). Transmission on-farm is possible via contaminated boots, needles, overalls and hands (Otake *et al.*, 2002b) and possibly semen (Prieto *et al.*, 1996). Virus has been transmitted from an inoculum containing boar semen to eight month old gilts (Prieto *et al.*, 1996). Experimentally infected boars have also tested positive for virus using PCR and virus isolation (Christopher-Hennings *et al.*, 1995). However, virus was inconsistently isolated from seminal plasma in one study (Christopher-Hennings *et al.*, 1995), which might suggest that transmission via semen might not be that common. Despite this evidence, it is thought that eight herds that became infected with PRRSV in the UK did so because they purchased semen from PRRSV positive breeding farms (Robertson *et al.*, 1992).

1.5.2 Clinical PRRS

The clinical signs of PRRSV infection include returns to oestrus, abortions and high pre-weaning mortality (Wensvoort *et al.*, 1991; Plana *et al.*, 1992). This has significant economic loss for farmers due to a reduction in sow productivity and

ultimately a lower number of pigs weaned. However, the disease is also a significant contributor to respiratory disease in post-weaning pigs (Drew, 2000). Clinical disease is variable between herds (Dee and Joo, 1994b; Baysinger, *et al.*, 1997; Bøtner, 1997). The virulence of the strain of PRRSV to which pigs are exposed has been hypothesised to affect the variety of clinical manifestations and varying viraemia that have been observed in the field, not only in different herds but also within a single herd (Mengeling *et al.*, 1996; Johnson *et al.*, 2004).

1.5.3 The immune response to infection with PRRSV

1.5.3.1 Humoral response

Pigs born to immune dams have maternal immunity until four to ten weeks of age in field studies (Albina, *et al.*, 1994; Houben, *et al.*, 1995; Nodelijk, *et al.*, 1997). Transmission is significantly lower amongst groups of pigs with maternal immunity, compared with groups of pigs without (Houben *et al.*, 1995; Nodelijk *et al.*, 1997). Following infection, PRRSV replicates in pulmonary alveolar and intravascular macrophages (Wensvoort *et al.*, 1992; Paton *et al.*, 1992). White blood cells and Immunoglobulin (Ig) M, IgA, IgG1 and IgG2 antibodies are produced in infected pigs (Labarque *et al.*, 2000) and positive Enzyme Linked Immunosorbent Assay (ELISA) results have been detected 12 - 15 days after infection (Johnson *et al.*, 2004). IgG is present for up to 49-52 days post infection (Labarque *et al.*, 2000; Joo *et al.*, 1997). However, the humoral response is not sufficient to eliminate the virus from individual pigs in some cases. The transfer

of antibodies from PRRSV-recovered sows to piglets has not led to the development of protective immunity (Lopez Fuertes *et al.*, 1999). In addition, virus is present up to seven weeks in macrophages, despite production of antibodies (Mengeling *et al.*, 1996).

1.5.3.2 Cell-mediated response

The cell mediated immune response is thought to be important in establishing protective immunity against re-infection with PRRSV. Cytotoxic T cells are present 4 – 11 weeks after infection in piglets (Bautista and Molitor, 1997) and are produced at a higher concentration and much sooner that at primary exposure with PRRSV. The presence of CD2+ and CD8+ cells and interferon gamma coincide with viral clearance from lungs of infected pigs (Labarque *et al.*, 2000; Batista *et al.*, 2004). Both are thought to clear virus and protect against reinfection, with CD8+ important in the control of virus replication and interferon-gamma vital in blocking macrophage infection with PRRSV (Albina *et al.*, 1998, Batista *et al.*, 2004).

1.5.4 PRRSV vaccination

Both live and attenuated vaccines are available for control of PRRSV. The efficacy of these vaccines is thought to be influenced by the degree of homology between the vaccine strain and the strain(s) present on-farm (Mengeling *et al.*, 2003b). Significantly lower viraemia was observed when pigs were vaccinated

and then challenged with a similar strain of virus, compared with subsequent challenge with a more different strain of virus (Labarque *et al.*, 2004). Concerns regarding the use of vaccine have stemmed from reports that virus still remains in serum six weeks after infection (Mengeling *et al.*, 2003a; Labarque *et al.*, 2004) and a non-reduction in viral infectivity titres in serum of vaccinated pigs has been observed, compared with non-vaccinated pigs challenged with a virulent viral strain (Mengeling *et al.*, 2003a). In addition, vaccination did not reduced viral replication or pulmonary lesions following infection (Mengeling *et al.*, 2003a) and vaccine strains have also crossed the placenta and infected developing embryos (Scortti *et al.*, 2006). Despite these observations, vaccination in the field has reduced the numbers of weak and unhealthy piglets (Plana-Duran *et al.*, 1997), and has reduced fever, lung lesions, viraemia (Mengeling *et al.*, 2003a) and reproduction losses (Papatsiros *et al.*, 2006). It has also been reported to increase the number of piglets weaned (Alexopoulos *et al.*, 2005).

1.6 Diagnosis of respiratory infection in pigs

Diagnosis of a respiratory infection can be done using serological, virological or bacteriological testing together with clinical signs, e.g. coughing (Straw *et al.*, 1990) or lung lesions at slaughter (Christensen and Mousing, 1992).

1.6.1 The enzyme- linked immunosorbent assay (ELISA)

ELISA is used to detect the presence and amount of antibody in a sample. Calibrated on a positive and negative control, the output from an ELISA is an optical density based on the amount of substrate present in the serum sample and the colour emitted when this binds with another substrate plus enzyme fixed within wells on the ELISA plate. The magnitude of the colour emitted from such a chemical reaction can indicate the concentration of antibodies present in the sample, compared with a positive control. These quantitative measures of antibody concentration can be used to determine time since infection because antibody concentration decays over time. Information on external risk factors can be used to investigate associations with higher antibody titres and more recent infection.

1.6.2 Age-specific serological surveys

Age-stratified cross-sectional data provide a snapshot in time of the proportion of individuals that have been exposed to a pathogen. Assuming constant transmission dynamics over time, an increase in the proportion of individuals that are seropositive between age groups can indicate age and / or time-specific risk factors (Ferguson *et al.*, 1999). Such data might also indicate age groups(s) that are at risk of infection (Gay, 1996). More detailed information about the environment can lead to the identification of associations between changes in seroprevalence and extrinsic factors that might influence the transmission dynamics of the pathogen being studied.

1.7 Hierarchical clustering of data

Populations of individuals are often clustered within a hierarchical structure, where individuals within the same level of the hierarchy are more alike, compared with those at a higher or lower level. Pigs are usually housed in pens of pigs of the same age. These pens are grouped in individual rooms within buildings and herds. Pigs within an individual cluster of this hierarchy often have characteristics in common, including age, management, feed, environment, immunity and exposure to an infectious pathogen. Multilevel modelling allows such clustering of individuals to be accounted for, attributing different levels of variance in the outcome variable to different levels of the hierarchy. Multilevel modelling has been previously applied to the modelling of infectious diseases in pigs. For example, Maes *et al.*, (1999) investigated the seroprevalence of infections in slaughter pigs in the USA and KilBride *et al.*, (2009) investigated pig, pen and herd-level factors associated with limb lesions in finishing pigs, gilts and sows in England.

1.8 Mathematical modelling of pathogen transmission dynamics

Mathematical models are representations of a system that involves interacting components, given certain parameters and rates. These systems can be used to

simplify reality, incorporating the main aspects of system functionality (Taylor, 2003). The uses of mathematical models include:

- Predicting the effects of changing different components of the system,
- To test (verify) and improve our understanding of a system,
- To analyse and explain behaviour of a complex system,
- To determine the relative importance of different components of the system (Taylor, 2003)

In infectious disease epidemiology, mathematical models are useful in understanding how an infectious disease might behave within a system (population), given certain components (i.e. susceptible, infectious and recovered individuals) and parameters (i.e. transmission rates, duration of infection and rate of recovery). Parameters determine the transitions of individuals between different states in the population, as defined by pathogen-specific characteristics. Outputs from such models are useful in projecting potential effects of transmission on a larger population scale (Keeling, 2005). They can also be used to observe the influence of demographic and immunologic change on disease dynamics, e.g. by isolation of individuals or by vaccination (Anderson and May, 1991).

Compared with deterministic models, stochastic models consist of random variability determined by probabilities of certain events occurring (MacKenzie and Bishop, 2001). This stochasticity is frequently introduced for the time at which the event occurs and which event occurs at that time point.

1.9 Aims of the thesis

The aim of this thesis was to investigate the associations between respiratory pathogens and morbidity and mortality in UK pig herds. The thesis is structured as follows:

- Chapter 2: investigation of the prevalence and incidence of the most common respiratory diseases in GB and the investigation of possible associations with one another and with post-weaning mortality
- Chapter 3: investigation of herd cross-sectional serology data for PRRSV and the determination of management factors associated with within and between-herd variability in pig antibodies
- Chapter 4: the development of a mathematical model of a typical pig farm in GB and the investigation of mechanisms for persistence and fade out of PRRSV using cross-sectional serological data
- Chapter 5: the impact of clinical disease due to PRRSV infection and its mitigation by different control and elimination strategies

Finally, I conclude with a discussion of what this research has achieved, how it has changed our understanding of respiratory disease in the UK pig population and what further research is required.

2 Chapter 2: Distribution of respiratory pathogens in 116 GB pig herds and their associations with post-weaning mortality

2.1 Background

Respiratory disease has been reported as the most important cause of morbidity and mortality in post-weaning pigs (Straw *et al.*, 1983; Losinger *et al.*, 1998). The presence of respiratory infection causes reduced growth rate in pigs (Huhn, 1970; Burch, 1982; Christensen *et al*, 1995), reduced feed conversion efficiency (Goodwin, 1971; Straw *et al.*, 1989) and increased post-weaning mortality (Losinger *et al.*, 1998).

Porcine respiratory disease complex (PRDC) is a term used to describe respiratory disease in pigs of 14-20 weeks of age, and involves several pathogens, including PRRSV, porcine circovirus type 2, porcine respiratory coronavirus, swine influenza virus (SIV), *Mycoplasma hyopneumoniae (M. Hyopneumoniae)*,

Actinobacillus pleuropneumoniae (A. pleuropneumoniae), Haemophilus parasuis (H. Parasuis), Pasteurella multocida and *Streptococcus suis* (Thacker, 2001). Simultaneous infection with more than one of these pathogens has been associated with more severe clinical disease than infection with one pathogen alone in individual pigs in experimental studies (Van Reeth *et al.*, 1996; Thacker *et al.*, 1999; Thacker *et al.*, 2001; Harms *et al.*, 2001; Rovira *et al.*, 2002; Opriessnig *et al.*, 2004).

There are many pathogens that cause respiratory disease. These are described briefly below. *A. pleuropneumoniae* is a gram negative bacterium that causes pleuropneumonia in pigs. Acute disease is associated with haemorrhage, fibrinous exudation and necrosis in the lungs and pleural cavity (Bosse *et al.*, 2002). Previous infection with *M. hyopneumoniae* might increase the susceptibility to *A. pleuropneumoniae* (Marois *et al.*, 2008). The presence of *M. hyopneumoniae* is often associated with the presence of other pathogens as well as poor environment or management (Ross, 1999). Infection can either be clinical or subclinical, with reduction in production efficiency and general respiratory disease (Ross, 1999). Compared with *M. hyopneumoniae* which is non-systemic, *H. parasuis* infection (Glasser's disease) is characterised by polyserositis, arthritis and meningitis (Amano *et al.*, 1994) and acute septicaemia (Peet *et al.*, 1983) and is associated with high mortality in young weaner pigs. Many respiratory infections are subclinical and pigs can often be carriers for a prolonged period of time. For SIV, this is not the case, rather it is associated with sudden respiratory disease, including coughing, fever and prostration, with fast recovery (Easterday and Van

Reeth, 1998). Lesions might be present or absent in the lungs and pre-existing pneumonia may cause high levels of mortality (Easterday and Van Reeth, 1998). Both PRRSV and PMWS have emerged since 1987. Whilst PRRSV is associated with reproductive disease in sows (Wensvoort *et al.*, 1991) and general respiratory disease in post-weaning pigs (Drew, 2000), PMWS is associated with severe wasting in pigs of 6-14 weeks of age, dyspnoea, enlargement of the inguinal lymph nodes, diarrhoea, pallor and jaundice (Rosell *et al.*, 1999). Porcine circovirus type 2 is thought to be the necessary agent, although not necessary the cause of PMWS (Turner *et al.*, 2009).

Pathogens are not necessarily randomly distributed between herds because different characteristics and management practices might determine whether a herd is infected with a pathogen. In addition, pathogens might cluster within a herd if the factors that are specific to introduction and / or persistence are the same. Pathogens might cluster between herds if herds share common risk factors for introduction. These might include pig movements or geographical location or the presence of certain pathogens that might indicate the likely presence of other pathogens. Farmers tolerate a degree of ill health in their stock, particularly if disease responds to treatment. However, when large numbers of animals are ill or die they will seek a diagnosis to ensure that the disease is managed effectively. This is typically via their veterinary surgeon, who will use a combination of their own skills and experience and laboratory investigations (pathology, biochemistry and microbiology) to make a diagnosis. Once a diagnosis is made in a herd, farmers and veterinarians rarely test for the same disease again because of the

expense. Consequently there is usually a date at which a disease was first known to be present on a farm.

Once in a herd, the presence of a pathogen might be constant, with that pathogen persisting in the pigs and / or environment, or sporadic, with spontaneous elimination and re-introduction. This is discussed further in Chapters 3 - 5. Detection of antibodies to a pathogen therefore does not necessarily indicate that the pathogen is on a unit because it might have spontaneously eliminated (faded out). In addition, the presence of antibodies in breeding sows could be due to exposure to the pathogen in a previous herd and these could be passed to young pigs in colostrum. However, antibodies on growing pigs, after the age at which maternal immunity has waned, do indicate that the pathogen is on the unit. It does not indicate that the pathogen is definitely causing disease without knowledge of current clinical signs that are pathognomic for that pathogen.

The aim of the current study was to use results from a questionnaire to veterinary surgeons who attended 116 pig herds in GB to determine the prevalence and incidence of six respiratory diseases and to determine their association between probable farm infection and post-weaning mortality.

2.2 Materials and Methods

2.2.1 Study design and population

Data used for this study were derived from the PMWS study at the University of Warwick; funded by DEFRA and The Meat and Livestock Commission (Woodbine *et al.* 2007). Data used here were obtained from responses to a self-administered questionnaire completed by veterinarians attending pig units that were visited by the research team; and also responses from on-farm interviews with unit managers during the farm visits. Stored serum samples were used to investigate antibodies to some of the pathogens listed above. The number of herds used in the different analyses described below is shown in Figure 2.1.

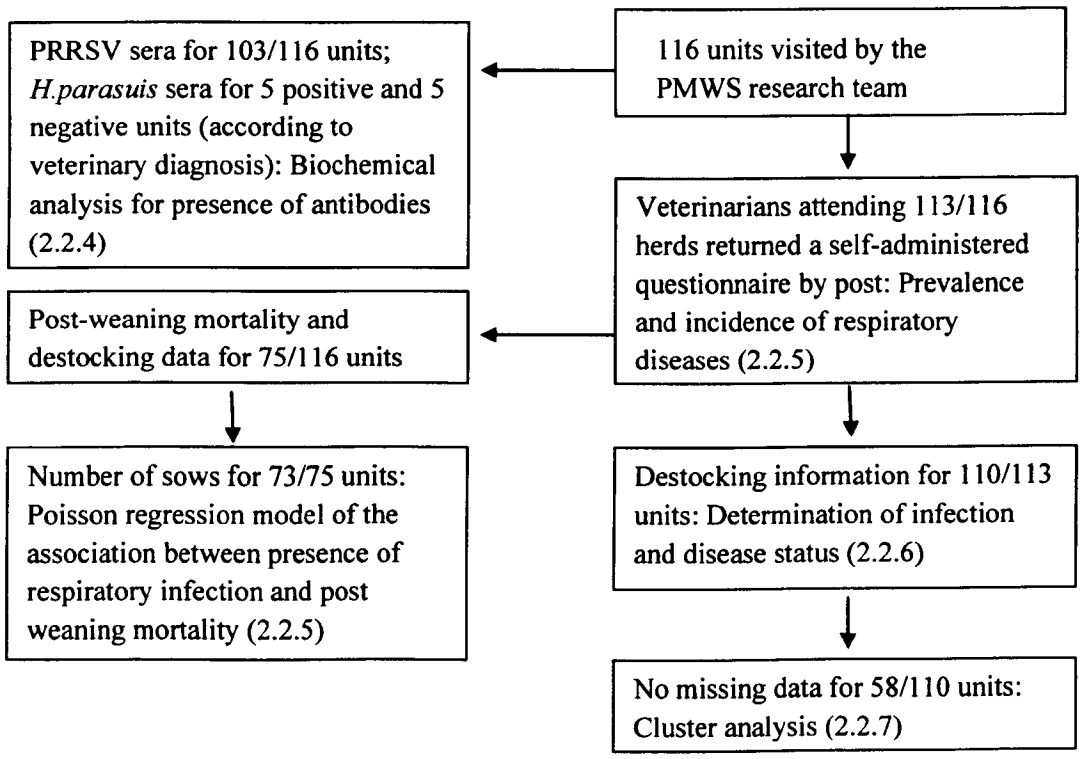


Figure 2.1 Flow diagram of the number of herds used for separate analyses based on available data

2.2.2 Questionnaire to veterinarians

In 2003-2004 with permission from unit managers, questionnaires were sent to veterinarians who attended the pig units that had been visited by members of the PMWS research group. Questionnaires were completed by 113 / 116 veterinarians who attended the pig units. The questionnaire was 10 pages in length and contained seven sections with a total of 78 questions. The majority of questions were closed unless exact figures were required. Data used in the current analysis were from responses to questions on the past history of disease caused by *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae*, SIV, PRRSV and PMWS on the unit. A section of the questionnaire is presented in Table 2.1. For each respiratory disease, the veterinarian was asked to state whether the disease had ever been observed on the unit, if yes, the year that the disease was first seen and how the disease was diagnosed. The veterinarian was also asked whether the unit was still affected with the disease, when clinical signs of the disease were last seen, current control strategies and whether disease from this pathogen had been exacerbated by the PMWS outbreak. Veterinarians were also asked to estimate the post-weaning mortality rate of the unit.

Disease	Ever seen on this unit (Y/N/DK)	Year first seen or >5 years ago?	Diagnosed by: Farmer (F) Self (S) Lab. (L)	Still on farm? (Y/N/DK)	Clinical signs last seen (yr or >5 yrs)	Current treatment or control	Increased during PMWS? (Y/N/DK)
e.g. Disease *A. pleuropneumoniae*	Yes	1998	S	Y	1998	Vaccinate	DK

Table 2.1 Example from the self administered questionnaire sent to veterinarians by post

2.2.3 On-farm interview with the unit manager

Unit managers were asked whether the unit had ever been destocked, when this took place and whether it had been partially or fully destocked. They were also asked how many sows were present on the unit at the time of the visit.

2.2.4 Biochemical analysis for presence of antibodies

From each of the 116 herds visited, 50 blood samples were collected: ten from pigs of both eight and 14 weeks of age and five from maiden gilts (breeding females in their first gestation) and five sows from each of parity one, two, three, four and five or older. Pigs of the same age were randomly selected from the same pen. Where there were insufficient numbers of pigs in a pen, those in adjacent pens were randomly selected. The serum was removed from the whole blood by

centrifugation by field technicians involved in the study and sera were stored at -20°C at the University of Warwick.

For this current study, sera were selected from 14 week old pigs from five herds that, according to veterinarians, had never had *H. parasuis* and five herds that were positive for *H. parasuis* (based on historical clinical diagnosis and recent serological testing). Selected sera were tested by the author at the University of Warwick for antibodies for *H. parasuis* using the Swinecheck ® HPS (Guildhay) ELISA in duplicate. All tests were performed according to the manufacturer's instructions and results were based on the positivity of the sample where S1 and S2 are samples in wells coated with and without antigen and P1 and P2 are positive controls in wells coated with and without antigen respectively:

$$\text{Ratio} = \left[\frac{OD_{405}S1 - OD_{405}S2}{OD_{405}P1 - OD_{405}P2} \right]$$

The sera from 103/116 herds visited were tested for PRRSV antibody at Leeds Veterinary Laboratory using the CIVTEST PRRS E/S SUIS (Hipra, Girona, Spain), a commercially available indirect ELISA with a sensitivity and specificity of 90.6% and 98.3% respectively according to the manufacturer. All tests were performed according to the manufacturer's instructions and results based on the

IRPC (relative index x 100) of the sample (equation shown) with a cut off of ≥ 20 determining seropositivity.

$$IRPC = \left[\frac{OD_{450}\,Sample - Mean\,OD_{450}\,Negative\,Control}{Mean\,OD_{450}\,Positive\,Control - Mean\,OD_{450}\,Negative\,Control} \right] x\,100$$

The presence and absence of seropositive 14 week old pigs was investigated and compared with veterinary opinion on whether PRRSV had ever been observed on farms that they attended.

2.2.5 Prevalence and incidence of respiratory diseases

Incidence rates were determined from veterinary responses to when a disease was first observed on a farm (Dohoo *et al.*, 2003), as follows:

$$Incidence\ rate\ (no.\ cases\ per\ 100\ herds\ per\ year) = \frac{Herds\ infected\ in\ year}{No.susceptible\ herds\ in\ population\ \ that\ year} x\ 100$$

2.2.6 Determination of infection and disease status

We assumed that infection was persistent in a herd if the veterinarian stated that clinical disease was present at the time of the questionnaire in 2003-2004 and the unit had not been depopulated since disease was last observed. Infection status

was assumed 'unknown' if the veterinarian did not know whether the disease had been observed or if disease had been observed in the past but the unit had been depopulated since it was last observed. Infection was assumed to be absent if disease had never been observed on the farm.

2.2.7 Cluster analysis

Cluster analysis was used to investigate the clustering of pathogens in individual herds. Infection presence or absence was represented by 1 and 0 respectively. Data were analysed using SPSS (14.0 for Windows) using Ward's clustering algorithm (Ward, 1963). The infection status of at least one infection was not known for 52/113 herds. Consequently these herds were added to clusters after the initial analysis when an individual infection was known to be present or absent. Log linear models were used to determine the significance of infections present and absent within individual clusters, using χ^2 analysis. The significance of clustering by veterinarian was investigated using χ^2 analysis.

2.2.8 Poisson regression model of the association between presence of respiratory infection and post-weaning mortality

The association between post weaning mortality rate and the presence of each of the six respiratory infections was investigated using a Poisson regression model in Egret (Egret 2.0.3 for Windows, Cytel Software Corporation). The number of breeding sows was used as the offset; and the outcome variable was the number of

deaths per 100 of the offset. Respiratory infections whose presence was significantly associated with mortality ($p \leq 0.1$) were tested in a final multivariable model.

2.3 Results

2.3.1 Response rate and information about the veterinarians

A total of 62 veterinarians attended the 116 units; 35.5% (22) were specialist pig practitioners and 85.5% (53) were members of the Pig Veterinary Society. Approximately 58.1% (36) attended 1-20 units in their practice, 25.8% (16) attended 21-100 units and 6.5% (4) attended 100 units or more. Approximately 35.4% of the units were considered, by their veterinarian, to be about average standard of management and 39.8% were considered to be better than average. Approximately 94.7% and 89.4% of unit managers had been given advice on the health of their herd by their attending veterinarian in 2003 and 2002 respectively. Veterinarians attending 107 units supplied information regarding how long the questionnaire took to complete and the average time spent was 56.5 minutes.

2.3.2 Prevalence and incidence of six respiratory diseases

According to veterinarians, 36.3% (41/113), 54% (61/113), 81.4% (92/113), 32.7% (37/113), 58.4% (66/113) and 81.4% (92/113) of herds had had disease attributable to *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae*, SIV,

PRRSV and PMWS respectively on units that they attended before 2003/2004. Only 3.5% (4/113) of the herds had not had disease attributable to any of the six pathogens. Of 113 herds, the number (%) that had previously had disease attributable to one, two, three, four, five or all six of the pathogens were 14 (12.4%), 15 (13.3%), 18 (15.9%), 33 (29.2%), 15 (13.3%) and 14 (12.4%) respectively (mean 3.4). Positive significant pairwise correlations were observed between *H. parasuis and A. pleuropneumoniae*, SIV and *A. pleuropneumoniae*, PRRSV and *H. parasuis*, PRRSV and *M. hyopneumoniae* and PRRSV and SIV ($p < 0.05$) (Table 2.2).

	App (41/113)	**Hp** (61/113)	**Mhyo** (92/113)	**SIV** (37/113)	**PRRSV** (66/113)	**PMWS** (92/113)
App (41/113)		32* (78%)	38 (92.7%)	22* (53.7%)	27 (65.9%)	39 (95.1%)
Hp (61/113)			51 (83.6%)	26 (42.6%)	41* (67.2%)	56 (91.8%)
Mhyo (92/113)				32 (34.8%)	62* (67.4%)	77 (83.7%)
SIV (37/113)					29* (78.4%)	33 (89.2%)
PRRSV (66/113)						61 (92.4%)

Table 2.2 Frequency table of past presence of pairs of the six infections based on clinical signs observed before 2003 / 2004

(*significant correlation at $p < 0.05$) (App – .A. *pleuropneumoniae*; Hp – *H. parasuis;* Mhyo – *M. hyopneumoniae*). Numbers in brackets within first row and first column indicate number of herds whose veterinarian stated that disease had been observed previously

The incidence rates of disease varied by pathogen (Figure 2.1). Clinical signs of *A. pleuropneumoniae* were not observed in these herds before 2000. Veterinarians then reported this disease in three herds in 2000 and 2001, in two herds in 2002 and in three herds in 2003. Overall the incidence was constant and low. The incidence of disease in herds due to *H. parasuis* peaked in 2002 (11.9 herds/100/yr were affected). This was also seen for PMWS (37.3 herds/100/yr). Most herds were affected by this time. Clinical disease due to PRRSV infection was observed as early as 1998 and continued to increase throughout 2000 and 2003, affecting 9.8 herds/100/yr in 2003. The highest incidence rate for disease due to *M. hyopneumoniae* (10 herds/100/yr) was observed in 1999. This remained at 8.7 herds/100/yr in 2004 (Figure 2.2).

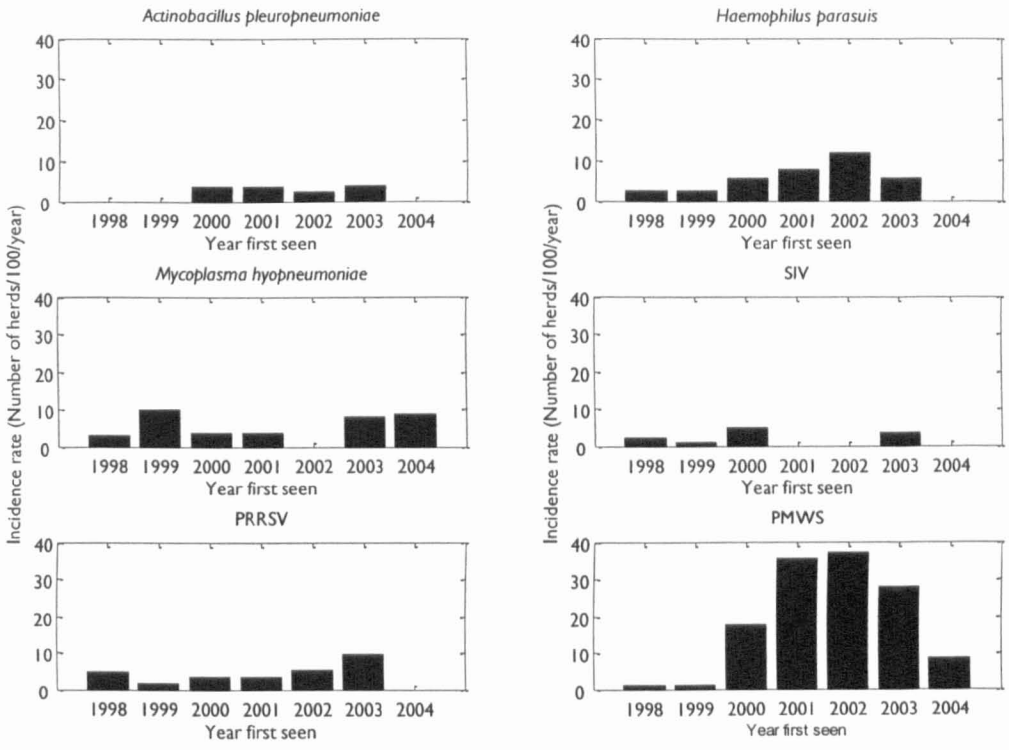


Figure 2.2 Time-dependent incidence rates of respiratory disease by pathogen and year

2.3.3 Herds in which infection was probably still persisting in 2003/2004

There was information on destocking for 110/113 herds. From this, infection was probably present in 32.7% (36/110), 50.9% (56/110), 77.3% (85/110), 27.3% (30/110), 51.8% (57/110) and 70.9% (78/110) herds for *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae*, SIV, PRRSV and PMWS respectively (Figure 2.2). In total, herds were infected with a mean of 3.1 pathogens in 2003/2004 (standard deviation 1.7). The number (%) of herds in which infection was present for 0, one, two, three, four, five and six of the pathogens was 9 (8.2%), 16 (14.6%), 14 (12.7%), 19 (17.3%), 28 (25.5%), 15 (13.6%) and 9 (8.2%) respectively.

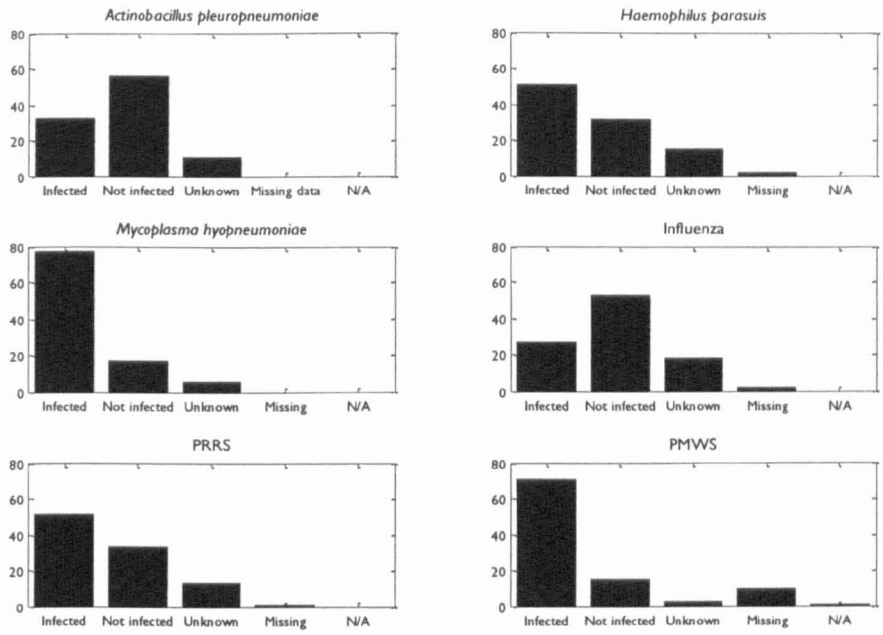


Figure 2.3 Percentage of herds in which infection was present in 2003 / 2004 by pathogen

Of 36 herds in which infection due to *A. pleuropneumoniae* was probably present in 2003/2004, 19 (52.8%) of the veterinarians reported that clinical signs had been observed in 2003 or 2004. Of 56, 85, 30, 57 and 78 herds in which *H. parasuis*, *M. hyopneumoniae*, SIV, PRRSV and PMWS were probably present in 2003/2004, 34 (60.7%), 62 (72.9%), 9 (30%), 37 (64.9%) and 71 (91.0%) veterinarians reported seeing clinical signs in 2003 or 2004.

For herds in which *A. pleuropneumoniae* was probably present in 2003/2004, 66.7% were using medication, antibiotics or vaccines in order to control infection and / or disease. Control measures were being used in 80.4%, 85.9%, 16.7%,

54.4% and 25.6% of herds in which *H. parasuis*, *M. hyopneumoniae*, SIV, PRRSV and PMWS were present respectively.

2.3.4 Presence of anti-PRRSV and anti-*Haemophilus parasuis* antibodies in sera by Enzyme Linked Immunosorbent Assay (ELISA)

Grower pigs were seropositive in 1 / 5 herds in which veterinarians stated that disease due to *H. parasuis* had been observed and in 5 / 5 herds in which veterinarians stated that disease due to *H. parasuis* had not been observed. This approximates to a sensitivity of 20% and a specificity of 0% respectively, based on serology as the presumed gold standard.

Grower pigs were seropositive in 21 / 25 herds in which veterinarians stated that disease due to PRRS had been observed. In 32 / 49 herds in which there were no PRRSV seropositive grower pigs, veterinarians reported never having seen PRRS on the unit, six did not know and 11 stated that the disease had been seen, three of which had been confirmed positive by laboratory diagnosis. Based on serology as the presumed gold stndard, the sensitivity and specificity of veterinary diagnosis for PRRSV was 20% and 0% respectively.

2.3.5 Cluster analysis

Fifty two of 58 herds for which disease status was estimated for all six pathogens, fell into distinct clusters (Table 2.3). Two of the five clusters were significant at p

<0.05 (Table 2.3). One of these clusters consisted of nine herds in which disease with all six pathogens was probably present and another cluster consisted of five herds in which only disease with *M. hyopneumoniae* was probably present (Table 2.3). These two clusters were still significant when herds with some missing data were included. The median number of herds attended by veterinarians was two (range 1 - 6). There was no evidence of clustering by veterinarian (p <0.05).

	Cluster 1*	Cluster 2	Cluster 3	Cluster 4	Cluster 5 *	Cluster 6	Cluster 7
	n = 9	n = 5	n = 9	n = 4	n = 5	n = 8	n = 12
A.pleuropneumoniae	+	+	-		-		
H.parasuis	+	+	+		-	+	-
M.hyopneumoniae	+	+	+	+	+		+
SIV	+			-		-	
PRRSV	+	+	+	+	-	-	
PMWS	+	+	+	-	-	+	+

Table 2.3 Cluster analysis of herds by pathogen present in 2003 / 2004 (+ / - indicates presence and absence of pathogen in all herds within the cluster, blank cells indicate pathogen presence for some herds within the cluster)

* Significant at 1 degree of freedom (p <0.05)

2.3.6 Post-weaning mortality and probable presence of respiratory pathogens in 2003/2004

Veterinarians attending 37 of the 116 units were unable to estimate post-weaning mortality rates and three unit managers did not supply destocking information from the on-farm interview, leaving 75 herds. For these 75 herds, post-weaning mortality ranged from 0.5% to 22% (mean 7.9%, median 7%). Median mortality

values for units in which 0, one, two, three, four, five and six infections were present were: 2.5%, 4.8%, 2.5%, 7.0%, 7.5%, 9.8% and 10.0% respectively (Figure 2.3). A χ^2 test for trend indicated significant increases in mortality with increasing numbers of infections ($p < 0.05$) (Figure 2.3).

Herds that probably had *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae*, PRRSV and PMWS in 2003 / 2004 had higher post-weaning mortality than herds not infected with these pathogens but these differences were not statistically significant. The absolute differences were 1.5%, 2.2%, 3.5%, 0.9% and 5.0% respectively. Mortality in herds that were unlikely to have SIV was 0.14% higher than herds that were probably infected. Non-significant differences in post-weaning mortality were observed between herds in which clinical disease had been confirmed by veterinarians since 2001, compared with those in which clinical disease had been observed before 2001 ($p > 0.05$).

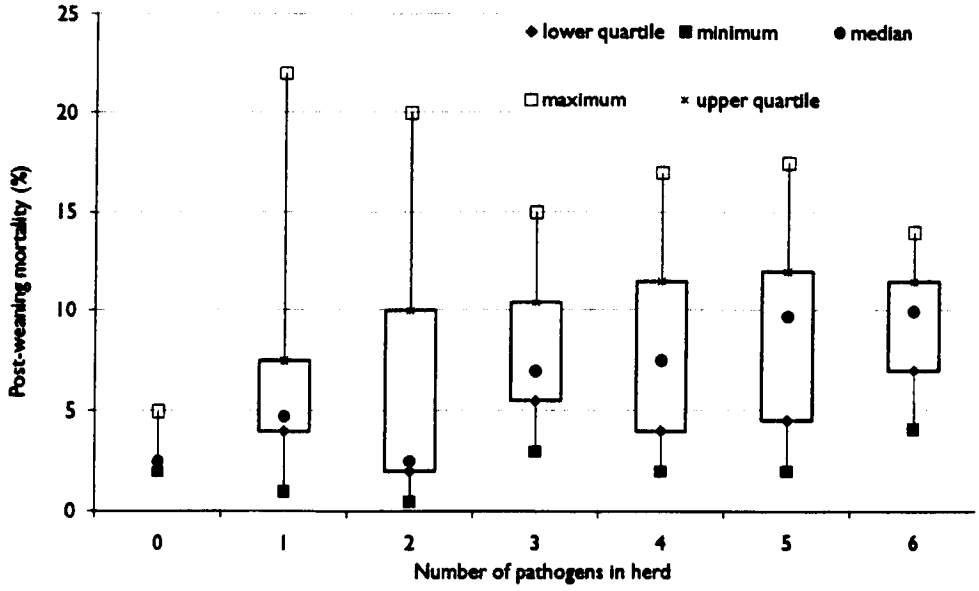


Figure 2.4 Post-weaning mortality and the number of pathogens present within herds in 2003 / 2004

2.3.7 Poisson models of the association of presence of pathogens within herds in 2003/2004 and post-weaning mortality

One herd did not have any breeding sows and another had missing data for the number of sows on the unit, leaving 73 herds. In the univariate screening, the presence of either *A. pleuropneumoniae*, *H. parasuis*, *M. hyopneumoniae* or PMWS were associated with higher post-weaning mortality rates at a significance level of $p < 0.05$ than those without these pathogens. The presence of PRRSV or SIV was not associated with higher post-weaning mortality rate. In the multivariable model, the presence of *M. hyopneumoniae* was associated with the highest post-weaning mortality, followed by *H. parasuis* and PMWS (Table 2.4).

	Coefficient	P value	Rate ratio	Confidence interval
Actinobacillus pleuropneumoniae				
Not infected (38)	Baseline			
Infected (29)	0.02	0.7	1.0	0.9 – 1.1
Unknown (6)	-0.2	0.02	0.8	0.7 – 1.0
Haemophilus parasuis				
Not infected (18)	Baseline			
Infected (42)	0.4	<0.05	1.5	1.3 – 1.7
Unknown (13)	0.4	<0.05	1.5	1.2 – 1.7
Mycoplasma hyopneumoniae				
Not infected (12)	Baseline			
Infected (57)	1.0	<0.05	2.6	2.2 – 3.1
Unknown (4)	0.5	<0.05	1.7	1.2 – 2.3
PMWS				
Not present (10)	Baseline			
Present (63)	0.3	0.3	1.3	0.8 – 2.1

Figure 2.5 Multivariable Poisson regression model of the association between presence of respiratory infections in 2003 / 2004 and post-weaning mortality

2.4 Discussion

This study investigated the presence of respiratory disease in 116 herds in GB, their associations with one another and with post-weaning mortality. Clinical history was used to determine presence of pathogens within herds and serology to validate the likelihood that veterinarians were knowledgeable of disease status. The advantage of using veterinarians is their knowledge of clinical signs of disease and results from abattoir surveillance and diagnostic tests. Veterinarians that participated in this study were generally experts on pig health issues, with approximately 35.5% who considered themselves specialist pig veterinarians and

85.5% members of the Pig Veterinary Society. However, the accuracy of veterinary diagnosis was low. In addition, a higher accuracy of veterinary diagnosis was observed for PRRSV, compared with *H. parasuis*. Differences in the accuracy of veterinary diagnosis would occur if disease presentation varied by pathogen, if infection with one pathogen can be mistaken for another or if laboratory tests are inaccurate for some diseases. It might also be possible for pathogens to differ in their transmission dynamics so that some do not persist following introduction.

Pathogens were assumed to be persisting on a farm unless depopulation had taken place since clinical disease was last observed. This assumption is based on the difficulty of eliminating pathogens without depopulation and observations that herds often become re-infected after depopulation and repopulation (Dee *et al.*, 1997). The risk factors for introduction are likely to remain unless a farmer changes these, and only some can be changed. In subsequent Chapters PRRSV antibody and therefore presence of infection within herds is modelled.

Pathogens were clustered within farms and were not clustered by veterinarian. This might suggest that there are risk factors for introduction and / or persistence that are common to more than one pathogen. These have been reviewed elsewhere and include herd size, air volume, stocking density, purchasing, production system and type, ventilation, bedding, pig movements, temperature and the distance to possibly infected farms (Stark *et al.*, 2000). The occasional presence of individual

pathogens, however, might highlight potentially different risk factors, or effective control or elimination.

The 116 herds sampled in this study were representative of the national herd in size, location and ratio of indoor to outdoor pig herds in 2004 (Woodbine *et al.*, 2008). However, farmer and veterinarian co-operation in the study might have been linked with the ongoing PMWS outbreak, as well as free serological testing (for parvovirus, PRRSV and PCV2) and monetary compensation for participation in this study. This sample of farms is therefore likely to include unit managers with both a concern for pig health and those that had health problems at the time of implementation of the study.

Some studies have reported non-significant differences in disease severity when single and multiple infections were compared (Alstine *et al.*, 1996; Pol *et al.*, 1997). This might suggest that additional factors play a role in clinical disease development, including the presence of other pathogens not investigated and / or environmental, management or genetic factors. Post-weaning mortality was highly variable in this study and ranged from 0.5% to 22%. The implementation of this study during the PMWS outbreak likely resulted in higher post-weaning mortality rates than reported in other studies, which have ranged from 0.7% (Losinger *et al.*, 1998) to 7.5% (Til and Dohoo, 1991) but interestingly were not linked to PMWS disease on the farm because it was present on most farms. Some variability in mortality was explained in the Poisson regression model by the presence of six

respiratory pathogens investigated here. However, post-weaning mortality was not significantly different between groups of herds that had / had not had clinical disease. This could be related to the small sample size in this study or other sources of variation of clinical disease between herds. Further studies are required to investigate the potential impact of these factors.

In the current study higher post-weaning mortality was associated with the presence of larger numbers of respiratory pathogens. However, the size of the incremental increases was greater when comparing infection with fewer pathogens. This might suggest that elimination of one pathogen from a herd might not make that much difference to mortality rates on-farm and only when a greater number of pathogens are eliminated would the decrease in mortality be large.

2.5 Conclusions

The apparent low accuracy of veterinary diagnosis has implications for effective understanding of respiratory infections within herds when used for research purposes and if it is a true low accuracy it also has implications for clinical management of these diseases. The study highlights the challenges of diagnosing multiple infections in one age group of pigs on one farm. The clustering of pathogens on-farm suggests that there are common risk factors for herd infection / persistence. However, the non-linear positive association between numbers of pathogens present on farms and post-weaning mortality highlight that elimination

of one pathogen from a herd might not significantly reduce clinical disease observed.

3 Chapter 3: Porcine reproductive and respiratory syndrome virus (PRRSV) in pig herds in GB: farm characteristics associated with heterogeneity in seroprevalence

The contents of this Chapter have been published (Appendix 1):

Evans, C., Medley, G. and Green, L. (2008). Porcine reproductive and respiratory syndrome virus (PRRSV) in GB pig herds: farm characteristics associated with heterogeneity in seroprevalence. *BMC Veterinary Research* **4**, 48.

3.1 Introduction

Porcine Reproductive and Respiratory Syndrome was first reported in North America in 1987 and in the UK in 1991 (Edwards *et al.*, 1992). Current estimates

are that 79% of breeder to finisher units in the UK are affected with PRRSV or are using vaccination (National Animal Disease Information Service, UK, 2007). The disease causes significant economic losses to the pig industry, costing approximately $560 million per year in the United States alone (Neumann *et al.*, 2005).

The clinical signs of PRRSV are reproductive loss in sows including return to oestrus, abortion, premature farrowing, mummified foetuses and stillbirths (Hopper *et al.*, 1992; Plana *et al.*, 1992). PRRSV causes high pre-weaning mortality in piglets infected *in utero* (Kranker *et al.*, 1998) and immunosuppression and consequent increase in susceptibility to other infectious diseases, particularly respiratory diseases in pigs infected post-weaning (Drew, 2000). The clinical disease caused by PRRSV is highly variable between farms. For example, whilst some seropositive herds have fairly consistent rates of respiratory disease (Stevenson *et al.*, 1993), others have periodic outbreaks of reproductive disease in breeding sows (Dee and Joo, 1994b) suggesting that the virus does not behave consistently between farms. There has also been a report of natural fade out of PRRSV on a farm (Nodelijk *et al.*, 2000) and some reports of active elimination of PRRSV from individual herds (Dee *et al*, 1993; Desrosiers and Boutin, 2002; Yang *et al.*, 2008).

The role of fade out and persistence in determining viral transmission dynamics has been recognised for some time, especially in the context of measles and other

childhood infections (Bartlett, 1957; Black, 1966). Periodic outbreaks of measles (and therefore episodes of fade out) have been observed in small communities (Bartlett, 1957), with low rate of supply of susceptible individuals (births) and low rates of virus introduction (Black, 1966). Persistence of a virus in a host population is critically determined by the availability (proportion) of susceptibles in the population, which is determined by, *inter alia*, transmissibility of the virus, infectious period and existence of alternative hosts or environment contamination (Anderson and May, 1992; Keeling and Grenfell, 1998, York *et al.*, 1979). Thus, for PRRSV, the observed variable clinical signs and natural fade out might occur because of variability in virus transmission within and between farms, different strains of virus, and / or because of transmission dynamic heterogeneity that results when most of the herd becomes immune.

Anti-PRRSV antibodies (detectable by ELISA) arise approximately 9 – 13 days after infection (Yoon *et al.*, 1995) and decay over time (Yoon *et al.*, 1995; Desrosiers and Boutin, 2002), persisting for up to 28 months (Desrosiers and Boutin, 2002). Most pigs clear virus within 3-4 months of exposure (Wills *et al.*, 2003), so most PRRSV antibody positive pigs are virus negative and consequently seropositivity is an indicator of past infection or vaccination. Whereas seropositivity of adult pigs might have been acquired many months previously in a herd in which the virus has become absent, seropositivity of young stock born on a farm indicates virus presence on that farm.

In this Chapter the farm and pig characteristics associated with herd seropositivity and pig heterogeneity in seroprevalence to PRRSV on 103 GB pig herds were determined. This was carried out using ELISA antibodies as a marker for previous exposure to PRRSV. Patterns of fade out and persistence are discussed.

3.2 Materials and methods

3.2.1 Study population and data collection

Data used in this study came from a cross-sectional study of 103 pig herds in England, Wales and Scotland. Data were collected from June 2003 to August 2004 as part of a study of PMWS (Woodbine *et al.*, 2007). From each herd, 50 blood samples were collected: 10 from pigs of both eight and 14 weeks of age and five from maiden gilts (breeding females in their first gestation) and five sows each of parity one, two, three, four and five or older. Pigs of the same age were randomly selected from the same pen. Where there were insufficient numbers of pigs, those in adjacent pens were randomly selected. The serum was removed from the whole blood by centrifugation and stored at -20°C. The sera were tested for PRRSV antibodies at Leeds Veterinary Laboratory using the CIVTEST PRRS E/S SUIS (Hipra, Girona, Spain), a commercially available indirect ELISA with a sensitivity and specificity of 90.6% and 98.3% respectively according to the manufacturer. All tests were performed according to the manufacturer's instructions and results based on the IRPC (relative index x 100) of the sample with a cut off of ≥ 20 determining seropositivity, where:

$$IRPC = \left[\frac{OD_{450}\,Sample - Mean\,OD_{450}\,Negative\,Control}{Mean\,OD_{450}\,Positive\,Control - Mean\,OD_{450}\,Negative\,Control} \right] x\,100$$

During the farm visit, the farmer was interviewed and management variables relating to the unit were recorded. Variables that were selected for use in the current analysis were plausibly associated with infectious disease transmission. These included the size and purpose of the herd, purchase of stock, quarantine facilities, biosecurity within the herd, and characteristics of the nearest pig unit (Table 3.1). In addition, the farmer's veterinarian completed a self administered questionnaire that included information on whether clinical signs of PRRS had ever been seen and if confirmed on the unit, when they were last seen, whether the veterinarian thought that the virus was still on the unit and whether pigs were vaccinated against PRRSV.

Herd attributes	Indoor or outdoor unit
	Nucleus or commercial unit
	Finisher site
	Number of sows (median 327, range 20 - 2300)
	Attending veterinarian specialist pig veterinarian
	Multiple site herd
	Pigs ever moved between sites
	Different system for sick pigs
	Sick pigs ever moved back to original batch group
	Purchased gilts mixed with sows
	Time after purchasing that gilts are mixed with sow group
	Presence of separate gilt housing
	Mixing of pigs with different batches
Purchased stock	Purchase gilts
	Purchase boars
	Purchase semen
Biosecurity	Presence of quarantine facilities
	Quarantine facilities on- or off-site
	Time incoming stock are isolated (median 6 days, range 0 - 28)
	Isolated stock exposed to other pigs in the herd
	Isolated stock tested for disease
	Semen tested for disease
	Protective clothing worn by employees
	Visitor pig-free time (median 48hrs, range 0 - 168)
	Footdip onto the unit and who this applies to
	Parking of vehicles on- or off-site
	Presence of a wheel dip onto the unit
Characteristics of nearest pig unit	Proximity of nearest pig unit (median 2 miles, range 0.1 - 17)
	Nearest pig unit indoor or outdoor unit
	Nearest pig unit nucleus or commercial unit
	Nearest pig unit finisher site
	Number of sows on nearest pig unit (median 250, range 0 - 2000)
Rodents	Birds observed in pig housing
	Rodents observed in pig housing

Table 3.1 Explanatory variables investigated in the statistical models obtained from the questionnaires with unit managers during farm visits June 2003 – August 2004

3.2.2 Data analysis

Seropositive pig – A pig was defined as seropositive if the IRPC of the sample was $\geq$ 20 units (according to manufacturer's instructions).

Seropositive herd – A herd was defined as seropositive if at least one pig in the herd was seropositive. Given the specificity of the ELISA (98.3%), a sample size of 50 pigs from a disease free population would result in an average of 0.85 positive pigs being detected. This definition minimises the false negative errors.

Vaccinated herd – A herd that, according to the veterinarian, was vaccinated. If the veterinarian did not give a response regarding vaccination, it was assumed that vaccination was not used.

FreeCalc (Version 2) (www.epiweb.massey.ac.nz) was used to calculate the minimum expected seroprevalence on a farm and by age when no seropositive animals were detected, adjusted by the sensitivity and specificity of the ELISA.

The total proportion of pigs seropositive per farm and for each age category was calculated and vaccinated and unvaccinated seropositive herds were compared. Seropositive herds were categorised according to whether there were any seropositive eight and 14 week old pigs (young stock) on the unit or not. The veterinarian questionnaire results were used to investigate the history of PRRS on all herds.

3.2.3 Statistical modelling

Three models were built.

Model 1. A binomial logistic regression model was used to determine associations between farm characteristics and the probability that a herd was seronegative for PRRSV antibodies. All veterinarians of vaccinated herds stated that PRRS had been seen on the units, so both vaccinated and positive herds were included in the model as seropositive herds. Analysis was carried out in Stata SE 9.0 (Stata Corporation, College Station, Texas).

Model 2. A 3 level mixed normal model was built in MLwiN version 2.1 (Rasbash *et al.*, 2000) to investigate the associations between quantitative IRPC values and herd-level predictor variables in seropositive herds, but where young stock were seronegative. The outcome was log (IRPC + 12) (12 was added to make all log values positive) and pig by pen by farm as the three clustered levels of the hierarchy. The fixed effects included age and management practices. The model took the form:

$$Log(ELISApositivity+12)_{ijk} = \beta X_0 + \beta X_k + \beta X_{jk} + v_k + \upsilon_{jk} + e_{ijk}$$

Where βX_0 is the intercept and βX is a series of fixed effect vectors that varied at the herd (k) and pen (j). v_k is the variance at herd level, υ_{jk} is the variance at the pen level and e_{ijk} is the variance of the log IRPC between pigs.

Model 3. Is as Model 2, but includes those farms where young stock were seropositive.

For all models continuous predictor variables were investigated for linearity with the outcome variable using five quintiles. The variable was transformed into a categorical variable if the relationship was not linear by eye. Pairwise correlated variables were identified using Pearson's pairwise correlation coefficient (for continuous variables) and χ^2 tests (for categorical variables), with Fisher's exact test where appropriate. To reduce the number of predictor variables for consideration in the multivariable models, significance for the univariable screening of variables was set at $p < 0.1$ (Dohoo *et al.*, 2003). Forward stepwise inclusion was used to build the multivariable models and confounding was assessed by evaluating the effect of the addition and removal of variables from the models. The significance probability for the multivariable models was $p < 0.05$. The model assumptions were investigated by observing distributions of pig standardized residuals; pigs that had significant influence on the model were determined by observing leverage values. Using pig-level seropositivity (absence or presence of antibodies) as a comparative outcome variable to log IRPC of pigs, the models were rerun with a binomial outcome and odds ratios were estimated for Models 2 and 3.

3.3 Results

A total of 4852 pigs from 103 herds were used in the analysis. Thirty five (34.0%) herds did not have any seropositive pigs, 41 (39.8%) had at least one seropositive pig and 27 (26.2%) were using vaccination (Table 3.2). The median herd size was 327 sows (range 20 - 2300). Based on 10 piglets born/sow/litter, two litters produced/year and an average slaughter age of six months (BPEX pig yearbook, 2006), approx. 3270 rearing pigs and 327 sows would be present on a median sized farm at any one time. If one or more seropositive pigs were detected from 50 pigs that were sampled per farm, the probability of the herd being truly seropositive is 95% at a prevalence of at least 12.2% (based on the sensitivity and specificity of the test).

Age of pigs	**Negative**		**Vaccinated**		**Positive**		**Total**	
	Herds	Pigs	Herds	Pigs	Herds	Pigs	Herds	Pigs
8 weeks	35	348	27	264	40	395	102	1007
14 weeks	34	339	27	256	40	396	101	991
Gilts	34	166	27	135	41	204	102	505
Parity 1	33	151	26	129	41	192	100	472
Parity 2	33	157	26	131	41	193	100	481
Parity 3	32	147	25	120	39	192	96	459
Parity 4	32	142	26	123	40	192	98	457
Parity 5+	32	160	26	126	40	194	98	480
Total	35	1610	27	1284	41	1958	103	4852

Table 3.2 Number of negative, vaccinated and positive herds and pigs in the study (4852 pigs from 103 herds in GB)

Vaccinated herds had a slightly higher seropositivity in adults and a similar seropositivity to 14 week old pigs in unvaccinated seropositive herds (Figure 3.1). Both vaccinated and unvaccinated positive herds had a significantly higher proportion of seropositive adults than herds with no seropositive young stock ($p < 0.05$).

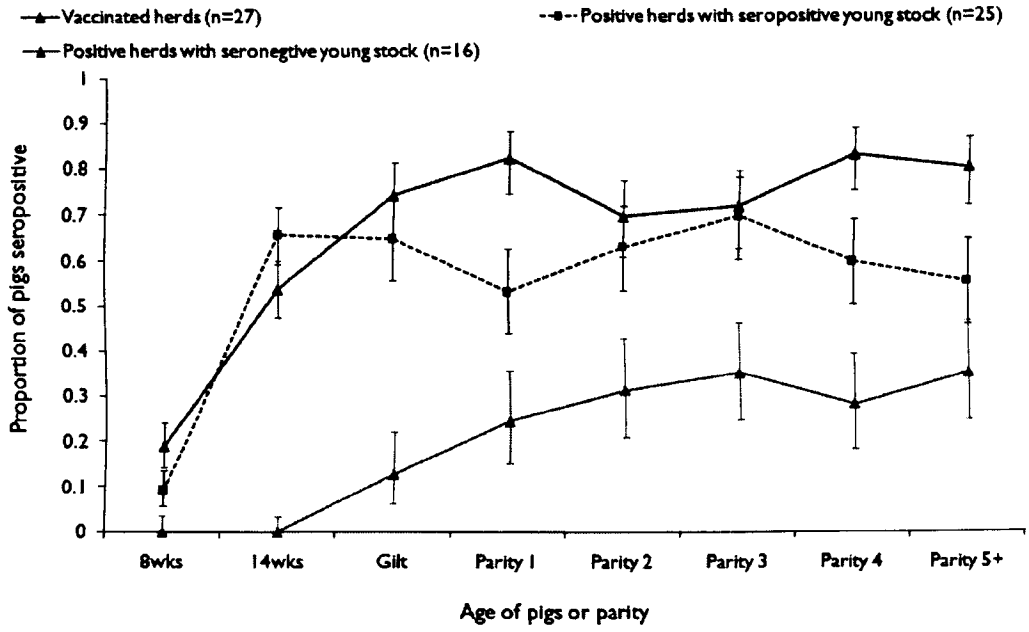


Figure 3.1 Proportion of pigs seropositive by age for 25 positive herds that had seropositive young stock, 16 positive herds that had seronegative young stock and 27 vaccinated herds

Bars indicate exact 95% confidence intervals for the proportion based on binomial distribution. Lines are included for ease of visual interpretation only.

There were 16 positive herds that had seronegative young stock (Figures 3.1, 3.2 and 3.3). A herd with an average of 3270 rearing pigs would have approximately 136 pigs of each week of age from 1-24. The total number of eight and 14 week

old pigs would therefore constitute 272 of the 3270 rearing pigs. A sample of 20 pigs from these age groups would be sufficient to detect a minimum seroprevalence of 22% with 95% confidence, given the sensitivity and specificity of the ELISA.

For the 16 positive herds that had seronegative young stock, different serological patterns were observed according to whether herds purchased gilts (10 herds – Figure 3.2) or only used homebred replacements (6 herds – Figure 3.3). For herds that used homebred replacements, older sows were more likely to be seropositive compared with younger sows (Figure 3.3). Two out of 10 herds that purchased gilts also had this pattern (Figure 3.2a, 3.2f) but for the majority of herds the seroprevalence was higher in younger sows on the farm (those purchased most recently) (Figure 3.2b-3.2e, 3.2g-3.2j) These individual farm age-seroprevalence curves demonstrate the between herd variability in exposure of pigs to PRRSV. There were no seropositive herds that had positive young stock and negative adults, nor were there more seropositive eight week old pigs compared to 14 week old pigs in any herds.

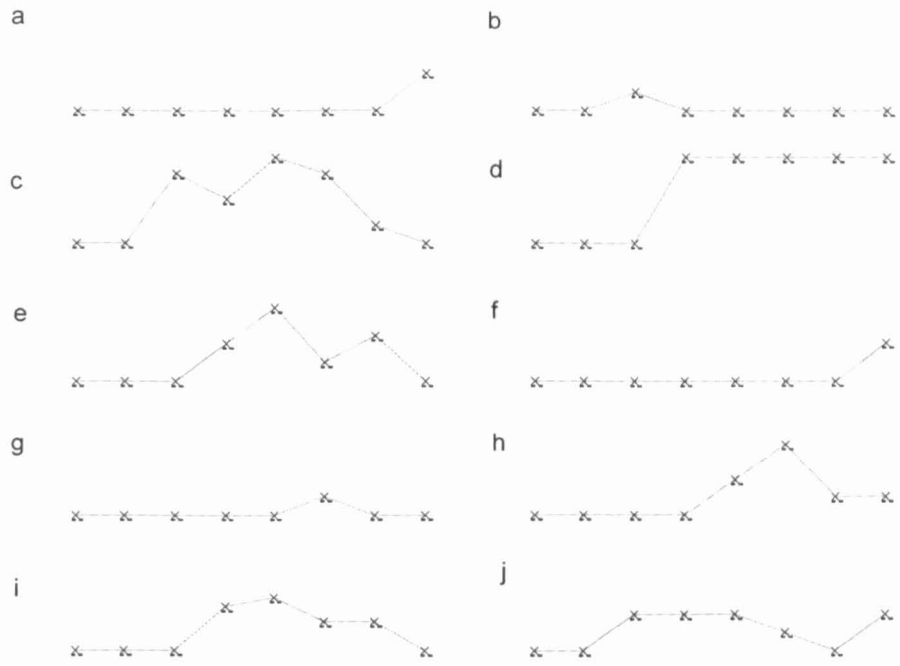


Figure 3.2 Proportion of pigs seropositive by age for ten seropositive herds that had completely seronegative young stock and purchased replacement gilts

x axis = Age of pigs or parity of sow (from left to right: 8 weeks, 14 weeks, gilts, parity 1, parity 2, parity 3, parity 4, parity 5+), y axis = Proportion of pigs seropositive (range 0 – 1).

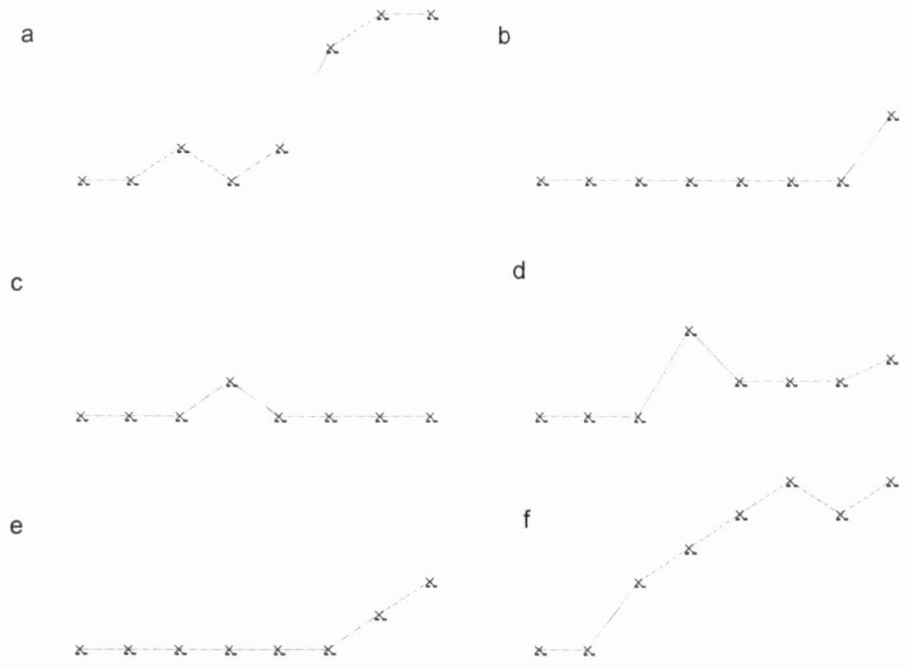


Figure 3.3 Proportion of pigs seropositive by age for six seropositive herds that had completely seronegative young stock and only used homebred gilts

(x axis = Age of pigs or parity of sow (from left to right: 8 weeks, 14 weeks, gilts, parity 1, parity 2, parity 3, parity 4, parity 5+), y axis = Proportion of pigs seropositive (range 0 – 1).

For 33 seronegative herds for which there were veterinarian responses, 26 reported never having seen PRRS on the unit and four stated that the disease had been seen, three of which had been confirmed positive by laboratory diagnosis. All veterinarians of the 27 vaccinated herds stated that PRRS had been seen on the unit in the past and 24 reported that PRRSV was still present. Of 25 seropositive herds with seropositive young stock, 21 veterinarians reported that they had seen PRRS in the past and 12 reported clinical signs since 2000. In addition, of 16 seropositive herds that had negative young stock, seven veterinarians had seen PRRS on these farms in the past and five reported clinical signs since 2002.

In the binomial logistic regression model (Model 1, Table 3.3), herds were more likely to be seronegative for PRRSV antibodies if there were <250 sows on the unit (OR = 3.86, 95% CI 1.46, 10.19) and if the nearest pig unit was situated >2 miles from the index herd (OR = 3.42, 95% CI 1.29, 9.12). A herd size of <250 sows was correlated with the unit being a nucleus or multiplier unit rather than a commercial unit. However, there were no other significant differences between commercial and nucleus or multiplier herds; therefore, the number of sows was included in the model. The nearest unit >2 miles away was correlated with the nearest unit not being a commercial unit (it was a nucleus, multiplier unit, hobby farm or an isolation unit).

Variable	Sample size	OR	SE	P	95% CI	
Herd size						
≥ 250 sows	75	reference				
<250 sows	27	3.86	1.91	<0.05	1.46	10.19
Distance to nearest pig herd						
<2 miles	40	reference				
≥2 miles	62	3.42	1.71	<0.05	1.29	9.12

Table 3.3 Model 1. Multivariable logistic regression model of factors associated with herds that were negative for PRRSV antibodies compared to those seropositive or vaccinated (103 herds in total)

Odds ratio (OR); standard error of the odds ratio (SE); probability function (p); 95% confidence interval (CI) (exp(coefficient+/-(1.96 x standard error)))

The mean log IRPC for pigs in seropositive herds was 3.02 (range, 0.83 – 5.58). In both Models 2 and 3, the log IRPC of pigs changed with age (Tables 3.4 and 3.5). In Model 2 (farms with seronegative young stock), the mean pig IRPC was 0.56 units lower when there were quarantine facilities on farm (95% CI -1.02, -0.10) and for every increasing mile distance between pig units there was a reduction in the log IRPC of 0.06 (95% CI -0.10, -0.01) (Table 3.4). The addition of the fixed effects accounted for 51.5% of herd-level variability. In Model 3 (farms with seropositive young stock), the mean pig IRPC was 0.61 units lower in herds that purchased gilts rather than used homebred replacements (95% CI -0.92, -0.29), 0.46 units lower when the farmer isolated incoming stock for $\geq$6 days (95% CI -0.81, -0.11) and 0.44 units lower if the statutory pig free time for visitors was $\geq$48 hours (95% CI -0.79, -0.10) (Table 5). The addition of fixed effects accounted for 64.8% of all herd-level variability. The model fit was good for both Models 2 and 3 and the assumptions of normality were reasonable. Variables significant in the final multilevel models were also significant when the binomial outcome variable (seropositive/seronegative) was used instead of pigs' log ELISA IRPC ($p < 0.05$) (Tables 3.4 and 3.5).

Variable	N	Coefficient	SE	p	CI[a]		OR	CI[b]	
Intercept		2.48	0.25						
Age category									
8 weeks		Reference					ref		
14 weeks	16	-0.06	0.2	0.76	-0.45	0.33	ref		
Gilts	16	0.52	0.2	0.01	0.12	0.92	ref		
Parity 1	16	0.93	0.2	<0.01	0.53	1.33	10.8	3.54	33.02
Parity 2	16	1.14	0.21	<0.01	0.74	1.54	16.3	5.44	48.84
Parity 3	16	1.19	0.2	<0.01	0.79	1.59	18.8	6.4	54.88
Parity 4	16	1.08	0.2	<0.01	0.68	1.48	12.72	4.21	38.41
Parity 5+	16	1.31	0.2	<0.01	0.91	1.71	8.39	2.87	24.56
Distance nearest pig herd (miles)	16	-0.06	0.02	<0.01	-0.1	-0.01	0.88	0.78	0.99
Quarantine facilities on farm									
Not present	4	Reference							
Present	12	-0.56	0.24	<0.05	-1.02	-0.1	0.27	0.08	0.87
Estimation of random effects:									
Variation (herds)		0.12	0.06						
Variation (pens)		0.29	0.05						
Variation (pigs)		0.24	0.01						

Table 3.4 Model 2. Multivariable three level mixed model of factors associated with log IRPC of 774 pigs belonging to 16 herds that had seronegative young stock

Sample size (n); ref (reference category); standard error (SE); probability function (p); 95% confidence interval (CI^a) when pigs' log IRPC (+12) values were used as a continuous outcome variable; Odds Ratios (OR) and 95% confidence interval (CI^b) when pig-level seropositivity (absence/presence of antibodies) was used as a comparative binary outcome variable (exp(coefficient+/-(1.96 x standard error))).

Variable	n	Coefficient	SE	P	CI[a]		OR	CI[b]	
Intercept		2.85	0.16						
Age category									
8 weeks	24	Ref							
14 weeks	24	1.47	0.14	<0.01	1.19	1.75	23.57	11.32	49.06
Gilts	25	1.52	0.15	<0.01	1.23	1.81	26.31	11.99	57.74
Parity 1	25	1.36	0.15	<0.01	1.07	1.65	13.37	6.14	29.11
Parity 2	25	1.52	0.15	<0.01	1.22	1.81	22.81	10.39	50.05
Parity 3	24	1.6	0.15	<0.01	1.3	1.9	29.93	13.32	67.25
Parity 4	24	1.38	0.15	<0.01	1.09	1.68	17.98	8.21	39.37
Parity 5+	24	1.42	0.15	<0.01	1.13	1.71	14.73	6.75	32.14
Purchased gilts									
No	13								
Yes	12	-0.61	0.16	<0.01	-0.92	-0.29	0.35	0.18	0.7
Length of time purchased stock isolated									
Not isolated	14	Ref							
1-5 days	3	-0.36	0.25	0.14	-0.85	0.12	0.61	0.21	1.77
6 days or more	8	-0.46	0.18	<0.01	-0.81	-0.11	0.43	0.2	0.93
Pig free time for visitors									
< 48 hours	17	Ref							
≥ 48 hours	8	-0.44	0.18	<0.05	-0.79	-0.1	0.44	0.2	0.93
Estimation of random effects:									
Variation between herds		0.11	0.04						
Variation between pens		0.2	0.03						
Variation between pigs		0.45	0.02						

Table 3.5 Model 3. Multivariable three level mixed model of factors associated with log IRPC of 1184 pigs belonging to 25 herds that had seropositive young stock

Sample size (n); ref (reference category); standard error (SE); probability function (p); 95% confidence interval (CI[a]) when pigs' log IRPC (+12) values were used as

a continuous outcome variable; Odds Ratios (OR) and 95% confidence interval (CI^b) when pig-level seropositivity (absence/presence of antibodies) was used as a comparative binary outcome variable (exp(coefficient+/-(1.96 x standard error))).

3.4 Discussion

The 103 herds sampled in this study were representative of the national herd in size, location and ratio of indoor to outdoor pig herds in 2004 (Woodbine *et al.*, 2007). Age-related antibody profiles were heterogeneous between farms, and much of the heterogeneity was explained by covariates that would be expected to be related to virus introduction (pig density, quarantine) or persistence (herd size). Although the data are from a cross-sectional study, the ELISA results indicate past exposure to PRRSV from which time-dependent patterns can be inferred. The prevalence of antibody positive pigs in one age group is not necessarily associated with the prevalence in another, because exposure to virus may have occurred at different times and, for sows, even on a different farm. The presence of antibodies in young stock indicates virus presence and transmission on that farm.

Classifying herds as virus negative on the basis of 20 seronegative eight or 14 week old pigs (irrespective of whether seropositivity was non-homogeneously distributed within the two age groups) is supported by three arguments. First, there was no passive immunity, which declines within 4-10 weeks (Houben *et al.*, 1995; Nodelijk *et al.*, 1997) indicating that sows were likely seronegative. Second, in the current study, unvaccinated herds with seropositive young stock had a mean seroprevalence of 67% in 14 week old pigs (Figure 3.1). When all 20

grower pigs were seronegative the true seroprevalence would be expected to be ≤22%, with 95% confidence. This suggests that young stock might act as sentinels for active virus transmission within a herd: they are either negative or highly positive. Third, both vaccinated and unvaccinated positive herds had a significantly higher proportion of seropositive adults than herds with no seropositive young stock, indicating that these herds had no / little active infection in adults.

The factors that may provide a pool of susceptible pigs and reduce the probability of herd immunity and so aid persistence of PRRSV on pig farms include production of susceptible piglets (approximately 22 per annum per sow) and the movement of pigs between farms, especially breeding stock, currently replaced at 45% per annum in the UK. These risks are correlated and decrease together as herd size decreases. There may be a threshold level when the probability of successful introduction reduces to below one. In the current study, this appears to be at ~ 250 sows. So a smaller herd size might reflect an increased probability of reduced risk of introduction of virus and / or virus fade out from lack of susceptible pigs. The association between fade out of PRRSV and herd size has been reported previously (Nodelijk *et al.*, 2000).

Herds with <250 sows were more often multiplier and nucleus herds. Such herds were also more likely to be situated >2 miles from the next nearest pig unit. It is not possible to state which of these factors, or the combination of factors, assists

seronegativity because these farms are more likely to be more biosecure than commercial farms and might be deliberately situated further away from the main pig breeding areas. Consequently, cause and effect relationships cannot be separated. However, biologically, a small population is more likely to lead to virus fade out.

PRRSV antibody negative herds were more prevalent when they were >2 miles away from the nearest pig unit. Seropositivity was also lower in herds that were more remote from other pig herds so local distant spread appears possible. The mechanisms by which virus may be transmitted between herds is currently not known, although aerosol transmission of PRRSV has been demonstrated over short distances (Brockmeier and Lager, 2002) and some birds and insects can harbour virus (Zimmerman *et al.*, 1997; Otake *et al.*, 2004) and so might transmit virus over longer distances. The association of lower IRPC values in positive herds when statutory pig-free time for visitors was >48 hours may reflect a possible route of introduction of virus, although not reported previously. It is also likely to correlate with generally high biosecurity. However the virus is transmitted, researchers in Denmark and the UK suggest that between-herd transmission of virus, not via pigs, is possible (Edwards *et al.*, 1992; Mortensen *et al.*, 2002).

As well as geographical isolation, purchase of known negative stock and quarantine of stock before introduction onto a farm may limit introduction of

PRRSV. Presence of quarantine facilities were not associated with antibody negative herds but were associated with herds that had all seronegative young stock, which are likely to have been virus negative (see below). In addition, time that purchased stock spent in isolation was associated with lower IRPC values in pigs in virus positive herds. This latter association could occur if pigs in isolation were more likely to be virus negative by the time they entered the unit. Isolation of new stock has been associated with a lower risk of introduction of PRRSV (Edwards *et al.*, 1992; Potter, 1994; Dee *et al*, 1994c).

A lower mean IRPC in positive herds with purchased gilts rather than homebred replacements would occur if there was a higher probability that purchased gilts were seronegative compared with homebred gilts: this probability would be high if the mean herd seropositivity was higher than the mean of all herds. Purchase of PRRSV negative gilts into a PRRSV positive herd might lead to disease in the herd if these gilts were infected when pregnant and this may explain some of the irregular disease patterns reported in positive herds (Dee and Joo, 1994b).

The presence of antibodies in breeding female pigs but not young stock has two possible explanations. First, gilts were exposed to virus or vaccinated on one farm and then introduced into a negative herd when they were seropositive but virus negative. Second, if virus had been transmitted on the farm in the recent past, but had since faded out, then it would leave seropositive virus negative older parity sows. This is seen in six herds that had seronegative young stock and used

homebred replacement gilts, where older sows were more likely to be seropositive. This suggests fade out of virus from the herd, since older pigs are more likely to have been present in the herd when virus was circulating. Following fade out of virus, younger pigs in the herd would not have been exposed to virus and so would remain seronegative. Four of these six herds that used homebred replacements had some seropositive gilts or parity one sows (suggesting that virus was present up until quite recently). This may suggest either the early stages of fade out (with younger pigs having not been exposed to virus) or the early stages of an outbreak, with virus in the breeding herd but not yet in the young stock. Two of the profiles of herds that had seronegative young stock and purchased gilts also suggest virus fade out, but the remaining eight had a higher proportion of seropositive younger sows compared with older sows. This suggests introduction of antibody positive stock into virus negative herds and a decline in the level of antibodies in older sows because they had been purchased, and presumably exposed, a long time previously and the level of antibodies had waned. These profiles suggest non re-exposure to virus after purchase and therefore a lack of virus in the recipient herd.

It is likely that both truly virus positive and truly virus negative herds that use homebred replacements are the most clinically and immunologically stable, since the former encourages active immunity in pigs before their first gestation and the latter have no virus. Introduction of viraemic stock into positive herds might assist in persistence of PRRSV through re-introduction and would be of concern if a different strain of PRRSV was introduced. Conversely, introduction of negative

pigs into a positive herd might cause disease because these gilts would be infected in their first gestation. Totally negative herds are at risk of PRRSV introduction if geographically close to another pig farm, of larger herd size or if purchasing and/or not isolating incoming stock.

Approximately 51.5% and 64.8% of the total between-herd variance (amongst seropositive herds) were explained in the two multilevel models respectively. As a result, the proportion of variation attributable to differences between pens and between pigs was high in both multivariable models. Approximately 37% and 59% of the total variation was attributable to differences between pigs in the two models respectively. The collection of data at the pen and pig levels may have accounted for some of this variability. Main sources of variability may include IRPC values between pigs (experimental error and strain variation) as well as presence of maternal antibody and the time of exposure to virus.

The decision to use ELISA log IRPC values as the continuous outcome variable in the multilevel models was based on <100% sensitivity of PRRSV ELISAs (Nodelijk *et al.*, 1996; O'Connor and O'Reilly, 2002). A binary outcome would have led to a possible misclassification if pigs with low PRRSV IRPC values were coded as seronegative when they were, in fact, low seropositive and vice versa. The normality of residuals and the similar pattern of significance of variables present in the multivariable models when the binary outcome was used imply the suitability of the data to this type of analysis.

3.5 Conclusions

Porcine reproductive and respiratory syndrome virus infection was far from consistent across this sample of farms, with herds ranging from seropositive pigs in all age groups, to seronegative in young stock and seronegative in all ages. The results suggest that PRRSV transmission dynamics exhibit viral fade out and re-introduction rather than indefinite persistence on infected farms. Whilst fade out may occur in smaller more geographically isolated herds with minimal introduction of infectious stock, persistence may be associated with large herds in pig-dense regions with continuous introduction of infectious stock. These results may explain why disease is variable between infected herds and indicate that different management strategies are required which depend on the current herd status

4 Chapter 4: A stochastic mathematical model of the within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV): fade-out and persistence

The contents of this Chapter are in press (Appendix 2):

Evans, C. M., Medley, Creasey, S. J., G. F., Green., L. E. (2009). A stochastic mathematical model of the within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV): fade-out and persistence. *Preventive Veterinary Medicine* **93,** 248 - 257

4.1 Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive-sense single-stranded enveloped RNA virus (Wensvoort *et al.*, 1991), in the family *Arteriviridae*, order *Nidovirales* (Meulenberg *et al.*, 1993). It was first discovered in North America in 1987 (Hill *et al.*, 1990). Despite large scale attempts to control and eliminate the virus, PRRSV remains an important cause of reproductive disease in sows (Hopper *et al.*, 1992), high pre-weaning mortality in piglets infected *in utero* (Kranker *et al.*, 1998) and respiratory disease in pigs infected post-weaning (Drew, 2000).

The main route of introduction of virus into British herds in 1992 was the purchase of breeding females from infected herds (Edwards *et al.*, 1992). Additionally, close proximity to infected herds, purchasing semen and larger herds increased the risk of infection (Edwards *et al.*, 1992).

Within-herd transmission of PRRSV occurs horizontally through nose-nose contact and vertically from sow to piglet *in utero* (Wensvoort *et al.*, 1991; 1992). Transmission is possible through urine and saliva (Wills *et al.*, 1997a), via semen (Prieto *et al.*, 1997), birds and insects (Zimmerman *et al.*, 1997, Otake *et al.*, 2004), contaminated clothes (Otake *et al.*, 2002b) and via aerosol up to a distance of approximately 120m (Pitkin *et al.*, 2009). Following introduction, the spread of

a virus through a herd is determined by the contact structure of pigs within the herd, i.e. the type, intensity and frequency of contacts (Lurette *et al.*, 2008).

Porcine reproductive and respiratory syndrome virus antibody patterns were analysed in a sample of 50 pigs selected from each of 103 pig farms in GB in a cross-sectional study (Chapter 3). Of these herds, 34% were seronegative. Of 40 positive, unvaccinated herds 39% had patterns indicative of fade-out of virus, where only breeding sows were seropositive (young rearing-pigs from these herds were all seronegative). Herd characteristics such as purchasing practices, isolation facilities, herd size and pig density in the region explained more than 50% of the between herd variability in PRRSV seropositivity. These results suggest that fade-out and re-introduction might be an important characteristic of PRRSV transmission dynamics.

Fade-out occurs when a virus becomes extinct in a population when the only infected individual recovers without transmitting infection. This is most likely to occur either early in an epidemic, when the number of infectious individuals is small or late in an epidemic when the number of susceptible individuals is small. Persistence (i.e. constant presence of virus) implies that the rate of supply of susceptible individuals and incidence of infection are balanced. Persistence and fade-out of a virus within a population is determined by the transmissibility of the virus, the infectious period, the host birth rate and the existence of alternative hosts or environment contamination (Yorke *et al.*, 1979; Anderson and May,

1992; Keeling and Grenfell, 1997). In the case of PRRSV, it is the birth rate of piglets and the replacement rate of breeding sows that will determine the probability of fade-out within a herd. An increase in the probability of fade-out in smaller herds has been reported for PRRSV (Nodelijk *et al.*, 2000) and analysis of cross-sectional serological data suggests that fade-out of PRRSV might be a relatively common phenomenon (Evans *et al.*, 2008).

If natural fade-out of PRRSV occurs in some herds, then it should be, theoretically at least, possible to control or eliminate the virus. However, natural fade out of the virus could leave the herd susceptible to re-infection and the impact of disease might be high if herd immunity was low.

A within-herd model of PRRSV transmission has been published (Nodelijk *et al.*, 2000), but did not include the contact structure of the pig herd with respect to age and reproductive status. Another model of a pig herd that investigated pig population dynamics included age structure (Lurette *et al.*, 2008), but the influence of such a structure on the transmission of PRRSV and its persistence and fade-out has not been investigated.

In this Chapter the within-herd transmission dynamics of PRRSV is investigated. The herd structure and demography are chosen to represent a typical farrow-finish pig herd present in Europe. Model parameters are drawn from the literature. The

model is used to investigate persistence and fade-out of virus, especially considering differences in isolation procedures, frequency of re-introduction of PRRSV, the contact structure within the herd and herd size. Cross-sectional serological field data are used to inform the transmission parameter for the model.

4.2 Materials and methods

All code was written and run in MATLAB® (Version, 7.0, MatLab, The MathWorks, Natick, MA, USA).

4.2.1 Model structure (demography)

The pig population within the herd was structured into four main groups within the model:

1) Sows with litters of piglets
2) A post weaning rearing group with pigs of 4-24 weeks of age
3) A gilt house with young replacement breeding females
4) A sow group with gestating breeding female pigs

Management cycles for each of the groups are depicted in Figure 4.1, where the arrows represent movements of pigs of the same age and / or reproductive state between groups in the herd. Pigs were moved at the end of each week. Individual batches of pigs remained together throughout their lifetime on the farm and their

contact structures were explicitly modelled on the basis of their position within the cycles, as described below.

4.2.1.1 Lactating sows

Sows were moved to the farrowing house one week before farrowing and remained for five weeks. Piglets remained in the farrowing house until they were four weeks of age before moving to the rearing herd.

4.2.1.2 The rearing-pig cycle

The rearing group was segregated into weaner (5-8 weeks of age), grower (9-16 weeks of age) and finisher (17-24 weeks of age) stages of production. Each week, 24-week-old pigs were moved to the gilt house as replacements or 'sent' to an abattoir: all batches of rearing-pigs then moved up the rearing herd housing to occupy vacant pens and a newly-weaned batch of piglets entered the weaner accommodation.

4.2.1.3 The gilt cycle

Gilts were either sourced from within the herd, or purchased into the herd from elsewhere. For sourcing within the herd, a batch of female finisher pigs was selected as replacement stock from the 24 week old pigs each week and joined the gilt group. After nine weeks in the gilt house, gilts joined the sow group at service

(at approx. 231 days old), and were defined as parity one sows, replacing sows that were culled.

4.2.1.4 The sow cycle

The sow cycle was 21 weeks long. It was assumed that sows spent four weeks in the service house, 12 weeks in the dry sow house and five weeks in the farrowing house. It was assumed that sows were served five days after weaning and were moved to the farrowing house seven days before farrowing (a gestation of 114 days). Sows went through this cycle a maximum of six times before they were culled from the herd. Sows of different parities at the same point in the cycle were assumed to be housed together.

It was assumed that 45% of breeding sows were culled each year (BPEX, 2008) after weaning (before service). The probability of a sow being served after each cycle was $\sqrt{(1-r)}$ where the replacement rate (r) was 45%. It was assumed that no parity one sows were culled. Sows going into their seventh parity were removed from the model. The longest period of time pigs could remain in the simulated herd was 43 months, or 1113 days (33 weeks when served, 21 weeks between consecutive services and six reproductive cycles in total).

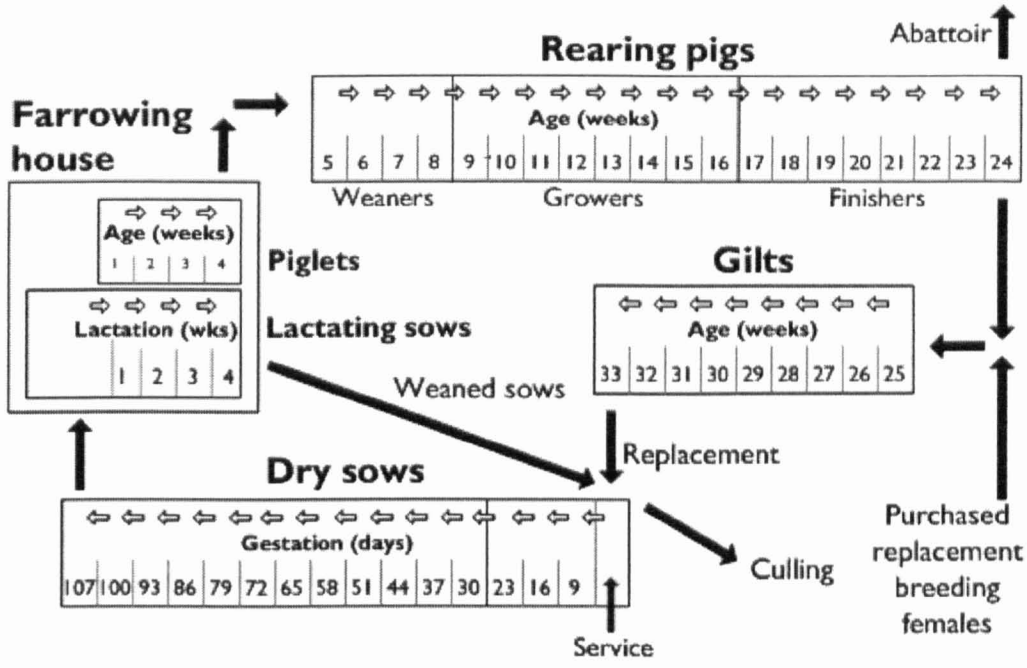


Figure 4.1 The structure of the demographic assumptions within the mathematical model

4.2.2 Epidemiological states and rate parameters

To simulate the transmission dynamics of PRRSV within the pig population, a stochastic Susceptible-Infected-Recovered-Susceptible (SIRS) model was used. Pigs belonged to one of five mutually exclusive states (defined below): passively immune (M), susceptible (S), infected (and infectious) (I), recovered and seropositive (immune and no longer infectious) (R_{pos}), and recovered and seronegative (susceptible to reinfection) (R_{neg}).

Transitions between states occurred at the rates given in Table 4.1. The times between events were chosen stochastically, assuming that all events were

independent. The three basic parameters determining the natural history of infection within individuals were assumed constant, and were estimated from published data as follows.

4.2.2.1 Rate of loss of passive immunity (π)

Piglets born to seropositive sows have passive immunity until 4-10 weeks of age (Houben, *et al.*, 1995; Nodelijk, *et al.*, 1997). Although PRRSV and antibody have been isolated from live piglets at birth (Botner *et al.*, 1994), high viraemic dose is required for transmission from infected piglets with maternal immunity (Houben *et al.*, 1995) and the majority of pigs are seronegative prior to entering finishing accommodation (Nodelijk *et al.*, 1997). If pigs were born viraemic, it was assumed that they also had maternal immunity and therefore contributed little or nothing to transmission of virus. In the model, the proportion of piglets born with passive immunity was equal to the proportion of infectious plus recovered (seropositive) dams farrowing that week and the rate of loss of maternal immunity (π) was 1/6 weeks.

4.2.2.2 Rate of recovery (α)

Transmission of virus to sentinel pigs has been demonstrated from pigs infected within the previous 56 days (Terpstra *et al.*, 1992) and in previous mathematical

models the most likely mean duration of infectiousness was 56 days (Nodelijk *et al.*, 2000). A recovery rate $\alpha = 1/56d$ was assumed.

4.2.2.3 Rate of loss of protective immunity (λ)

After recovery, pigs were assumed to be immune to further infection. In the field, pigs can become seronegative following recovery. This occurs 4.5 - 20 months after initial exposure (Yoon *et al.*, 1995, Desrosiers *et al.*, 2002). In the model, pigs became seronegative with rate $\lambda = 1/252$ days (9 months), and could be re-infected.

Event	Rate	Transition
Transmission to susceptible pigs in batch *i*	$\Lambda(i)S(i)$	S = S -1 I = I + 1
Recovery	αI	I = I -1 $R_{pos} = R_{pos} + 1$
Loss of passive immunity	πM	M = M -1 S = S + 1
Loss of protective immunity	λR_{pos}	$R_{pos} = R_{pos}$ -1 $R_{neg} = R_{neg} + 1$
Reinfection of recovered seronegative pigs in batch *i*	$\Lambda(i)R_{neg}(i)$	$R_{neg} = R_{neg} - 1$ I = I + 1

Table 4.1 Rates of transitions of pigs between different infection states in the model

S = susceptible, I = infected (and infectious), M = maternally immune, R_{pos} = recovered and seropositive (immune and not infectious), R_{neg} = recovered and seronegative (susceptible to reinfection), N = number of pigs, β = transmission parameter, α = rate of recovery (1/56 days), π = rate of decay of maternal immunity (1/6 weeks), λ = rate of decay of protective immunity (1/252 days). The rate of infection, $\Lambda(i)$, is given in eqn. (1).

4.2.3 Transmission parameters

4.2.3.1 Transmission of PRRSV in-utero

Experimental infection of sows on or after day 84 of gestation causes late-term abortions and the birth of stillborn and mummified pigs (Wensvoort *et al.*, 1991, Kranker *et al.*, 1998). Infection before this period has led to conflicting probabilities of *in utero* infection (Christianson, *et al.*, 1993 and Kranker, *et al.*,

1998) and in the field, the probability that infected sows abort and the probability of *in utero* death are not known. In the model, it was assumed that if sows were infected on or after week 12 of gestation (day 84), they had a 10% probability of aborting: the remaining 90% were assumed to farrow and give birth to seven alive piglets and four piglets that were either born dead or die prior to weaning. Sows that aborted were assumed to be undetected by the farmer and went through the cycle as normal, but the number of piglets born each week was reduced accordingly.

4.2.3.2 Horizontal transmission

The pig herd was divided into discrete batches determined by week and grouping (Figure 4.1). The rate of infection of susceptible individuals in batch *i* was calculated as:

$$\Lambda(i) = \beta \sum_{j} \frac{\phi(i,j) I(j)}{N(j)}$$

where I(j) and N(j) are, respectively, the numbers of infectious pigs and total pigs in batch *j*, β was the overall transmission parameter and $\phi(i,j)\beta(i,j)$ was the relative rate of transmission from infectious pigs in batch *j* to susceptible pigs in batch *i* (Table 4.2). This formulation assumed density independent transmission

(i.e. transmission did not increase linearly with the density of pigs in batches) and equal random mixing between pigs in the same batch.

The relative rates of transmission were simplified by assuming equal random mixing within dry sows and within maiden gilts. The rate of transmission between pigs in pens that were closely situated to each other including the weaner, grower and finisher pens within a house and between batches of sows in the service house was 1×10^{-3} lower than between pigs within the same pen. The rate of transmission between pigs in buildings that were closely situated to one another (i.e. batches of sows farrowing in the same week in the farrowing house, between maiden gilts and gestating sows, between maiden gilts / gestating sows and sows in the service house and between weaner, grower or finisher houses) was 1×10^{-4} lower than between pigs within the same pen. Transmission between sites within the herd, including the rearing herd, the farrowing house and the breeding herd was 1×10^{-5} lower than between pigs within the same pen.

Relative transmission parameters were changed in the model to simulate different contact structures. Without isolation, the relative transmission parameter between maiden gilts and gestating sows was increased to one for equal random mixing. Increasing the transmission parameters between pens in individual houses within the rearing herd to 0.1 increased contact between rearing-pigs, and increased contact between all pigs in the herd was achieved by increasing all relative transmission parameters by a factor of 100 (up to a maximum of one).

4.2.4 Comparison of model with data

Cross-sectional serological profiles of 40 positive non-vaccinated herds from a previous study (Evans *et al.*, 2008) were used to estimate the transmission parameter. The median herd size of the 40 herds was 343 sows (range 60, 1500). The herds were categorised by herd size into seven groups: 0-150, 151-300, 301-450, 451-600, 601-750, 751-900 and 1500 sows. The observed data are summarized in Table 4.3. For each herd, h, a number of pigs was randomly selected from eight age groups, n(h,a) (h = 1…40, a = 1..8) of which p(h,a) were positive. The test used to determine seropositivity was CIVTEST PRRS E/S SUIS (Hipra, Girona, Spain), a commercially available indirect ELISA with a sensitivity and specificity of 90.6% and 98.3% respectively (according to the manufacturer).

	Maiden gilts	Sows (service)	Sows (gestating)	Sows (same farrowing room)	Sows (different farrowing rooms)	Rearing pigs (same pen)	Rearing pigs (same house)	Different buildings
Maiden gilts	1							
Sows (service)	0.0001	1						
Sows (gestating)	0.0001	0.0001	1					
Sows (same farrowing room)	0.00001	0.00001	0.00001	0.001				
Sows (different farrowing rooms)	0.00001	0.00001	0.00001	0.0001	0.0001			
Rearing pigs (same pen)	0.00001	0.00001	0.00001	0.00001	0.00001	1		
Rearing pigs (same house)	0.00001	0.00001	0.00001	0.00001	0.00001	0.001	0.001	
Different buildings	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.001	0.0001

Table 4.2 Matrix of relative cross transmission probabilities of PRRS virus between different groups of pigs in the model

Herd	8 weeks	14 weeks	Gilts	Parity 1 sows	Parity 2 sows	Parity 3 sows	Parity 4 sows	Parity 5+ sows
1	0/10	0/10	0/5	0/5	0/5	0/5	0/5	2/5
2	0/10	0/10	1/5	0/5	0/5	0/5	0/5	0/5
3	0/10	0/10	4/5	2/4	1/1	4/5	1/5	0/4
4	0/10	0/10	0/5	5/5	5/5	5/5	5/5	5/5
5	0/10	0/10	0/5	2/5	4/5	1/5	1/2	0/5
6	0/10	0/10	0/5	0/5	0/5	0/5	0/5	2/5
7	0/10	0/10	1/5	0/5	1/5	4/5	5/5	5/5
8	0/10	0/10	0/5	0/5	0/4	1/5	0/4	0/5
9	0/7	0/10	0/5	0/5	0/5	0/5	0/5	2/5
10	0/10	0/10	0/5	0/5	2/5	4/5	1/5	1/5
11	0/10	0/9	0/5	1/2	3/5	2/6	2/6	0/5
12	0/9	0/10	0/5	1/5	0/4	0/5	0/4	0/5
13	0/10	0/10	2/5	2/5	2/5	1/5	0/4	2/5
14	0/10	0/10	0/4	2/4	1/5	1/5	1/5	1/3
15	0/10	0/10	0/5	0/4	0/5	0/4	1/5	2/5
16	0/10	0/10	2/5	3/5	4/5	5/5	4/5	5/5
17	1/10	0/10	0/5	0/3	0/5	0/5	0/5	0/5
18	0/0	0/0	0/5	1/5	2/5	0/0	0/0	0/0
19	0/10	1/10	1/5	0/4	2/4	3/4	1/5	1/4
20	1/10	1/10	4/5	3/5	5/5	5/5	3/5	5/5
21	0/10	2/10	0/5	1/5	0/5	0/5	0/5	1/5
22	2/10	2/10	5/5	2/5	4/6	4/4	5/5	5/5
23	0/10	4/10	4/5	2/5	5/5	3/5	4/5	4/5
24	0/10	6/10	4/5	1/2	1/4	2/4	2/5	0/5
25	0/10	6/10	5/5	3/6	2/4	4/5	5/5	1/5
26	1/10	6/10	1/5	0/5	1/5	0/0	1/4	1/5
27	1/10	5/8	5/5	4/5	5/5	5/5	4/5	5/5
28	0/10	7/10	5/5	2/5	4/5	3/5	2/5	1/5
29	3/10	7/10	3/5	4/5	4/5	2/5	4/5	2/5
30	0/10	8/10	2/5	1/2	2/5	3/5	0/5	0/5
31	1/10	8/10	3/5	3/5	3/5	5/5	5/5	5/5
32	2/10	8/10	4/5	4/5	4/5	5/5	5/5	4/5
33	0/10	9/10	5/5	5/5	2/5	4/5	1/5	0/5
34	0/10	10/10	2/5	3/5	5/5	5/5	3/5	2/5
35	0/10	9/9	5/5	3/7	2/3	5/6	2/4	5/5
36	0/10	10/10	4/5	4/5	3/3	0/4	4/5	0/3
37	0/10	10/10	4/5	4/4	4/5	5/5	5/5	5/5
38	0/10	10/10	5/5	3/5	4/5	5/5	4/5	5/5
39	2/10	10/10	4/5	4/5	4/5	4/5	3/5	5/5
40	7/10	10/10	5/5	5/5	5/5	4/5	5/5	5/5

Table 4.3 Number of pigs positive for PRRSV antibodies by ELISA / number of pigs sampled. Field data of 40 herds (collected during 2003 – 2004) were used to inform the transmission parameter for the mathematical model

The median proportion of seropositive pigs was 0 for eight week old pigs, 0.2 for 14 week old pigs, 0.4 for gilts, parity one and parity two sows, 0.6 for parity three sows and 0.4 for both parity four and parity five or older sows

In the model, pigs in the infectious, maternally immune and positive recovered (I, M and R_{pos}) states were considered as seropositive, i.e. they had sufficient antibody concentration that a perfect serological test would disclose them positive. Pigs in the susceptible and negative recovered (S, R_{neg}) states were considered seronegative. The model probability distribution of selecting truly seropositive pigs from a sample equal to the denominator in the data was calculated as binomial with the following probability per pig:

$$\frac{I(i)+M(i)+R_{pos}(i)}{N(i)}$$

The probability distribution of u positive tests from a sample of v pigs in batch i, Q(u,v,i) was then translated into a probability distribution of test positive by convoluting all the possible combinations of pig states and test outcomes. To compare the model output with the data, the probability distribution of test positive outcomes was used to calculate a herd level log-likelihood, which was summed across model repetitions to obtain an average model fit statistic:

$$L(h) = \sum_{reps} \sum_{a=1}^{8} \log Q(p(h,a), n(h,a), a)$$

The model was run for 1000 repetitions over 1200 days and the log-likelihood above computed every 21 days. One thousand simulations were sufficient to capture the range of model outcomes. Transmission parameters that maximised the likelihood values of the model outputs (given the data) were used.

The maximum log-likelihood for individual herds over 1200 days (described above) gave the most likely time that a particular cross-sectional serological profile could be generated during a simulated epidemic. Maximum likelihood of the model compared with all 40 herds from the field study indicated most likely times since introduction of virus and relative states of transmission dynamics.

4.2.5 Initial conditions

Before virus was introduced, the model was run for 1000 weeks to ensure demographic equilibrium. One infectious gilt was introduced into the gilt house (within a batch) and all other pigs in the herd were susceptible.

Following single introduction of a single infected gilt into the herd, virus could be re-introduced by changing the probability of gilts being infectious upon

replacement. In these scenarios, it was assumed that no gilts were sourced from within the herd. A range of 0.0025 to 0.04 was used, which represented 1 / 400 gilts to 1 / 25 gilts being infectious. Fade-out occurred when the number of infectious pigs in the herd was zero and a histogram of time to fade-out was constructed for each set of initial conditions.

4.3 Results

4.3.1 Cross-sectional field data

The maximum likelihood of the model outputs, given the cross-sectional serological field data and the sensitivity and specificity of the ELISA, varied with time since introduction of virus (Figure 4.2). During the early stages of the epidemic, likelihood values of the model outputs either steadily increased initially post-introduction and then remained constant, or showed a marked peak soon after introduction. That is, the serological profiles indicated either persistence or fade-out. The peak in likelihood was <6 months post-introduction for 8/40 of the seropositive unvaccinated herds from the field study, 6-12 months for 8/40 of the herds, 12-24 months for 9/40 of the herds and >24 months for 15/40 of the herds (Figure 4.2). Herds with seropositive 14 week old pigs (from the observational data) were more likely to have had virus for a longer period of time at the time of blood sample collection, although the presence of seropositive eight week old pigs in the model was usually associated with more recent introduction of virus (Figure 4.2). Where only adults were seropositive (from the data) the log-likelihood

peaked earlier on in the simulated epidemics unless they only had seropositive older sows, in which case log-likelihood profiles also peaked later, consistent with the latter stages of fade-out of virus.

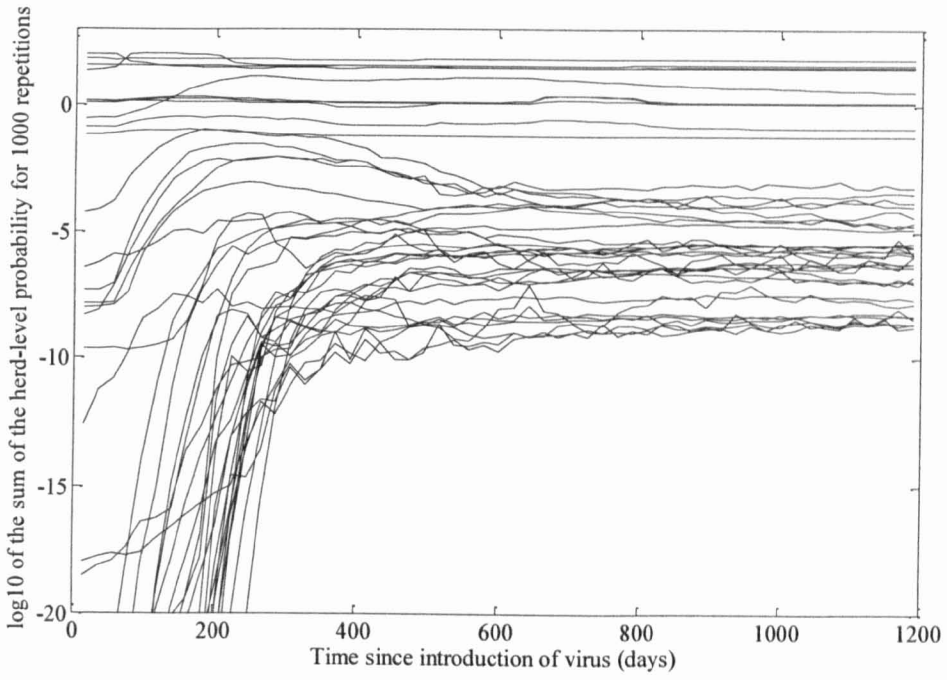


Figure 4.2 Log likelihood of the model outputs at 21 day intervals with time since introduction of PRRS virus, given the cross-sectional field data from 40 herds

4.3.2 Within-herd transmission dynamics following single introduction of virus

Using model parameter values that were most consistent, and assuming a herd size of 327 sows (the median herd size of herds in the field study), fade-out of virus occurred before 63 days in approximately 50% of repetitions, when virus was present in the gilt group only. Similarly, assuming that sows spent four weeks

in the service house and 12 weeks in the dry sow house in total, virus was restricted to the breeding gilts and sows in approximately 77% of repetitions (770/1000). If virus did not fade-out within 175 days of introduction, it was unlikely to fade-out within 1200 days (the duration of time the model was run). This corresponds with virus being unlikely to fade-out once it has reached the farrowing house and rearing-pigs.

4.3.3 Isolation and contact structure

When replacement gilts were assumed to mix equally with breeding sows before service (no isolation), the probability of fade-out soon after introduction was lower compared with when gilts were isolated from sows (Figure 4.3). When isolation was assumed, 81.6% of simulations resulted in fade-out within 250 days compared with 14.3% without isolation. The probability of virus persisting for >1200 days increased from 17.6% to 23.8% when rearing-pigs in different pens within one house transmitted virus to one another with a probability of 10% (instead of 0.1%) (Figure 4.4). In addition, the probability of persistence was 57.7% when cross-transmission terms between all groups of pigs in the herd were increased 100 fold (Figure 4.4).

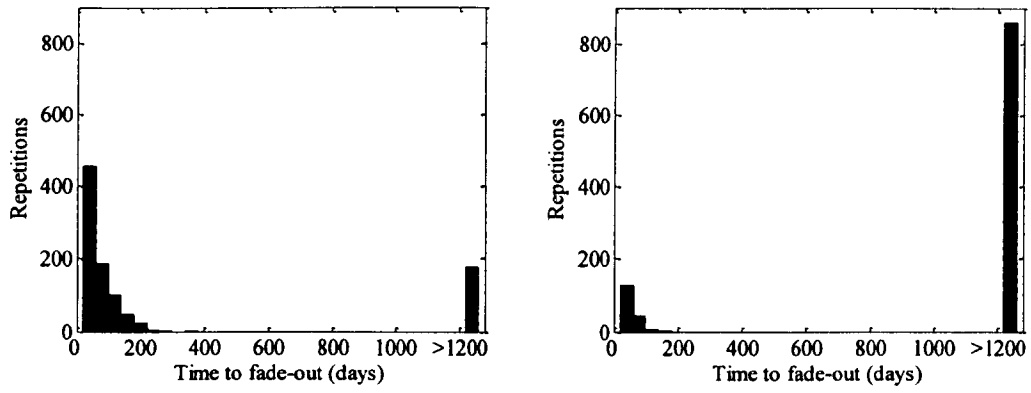


Figure 4.3 Time to fade-out of PRRSV for differences in isolation practices following introduction of one infectious gilt (herd size 327 sows)

Left: isolation, right: no isolation

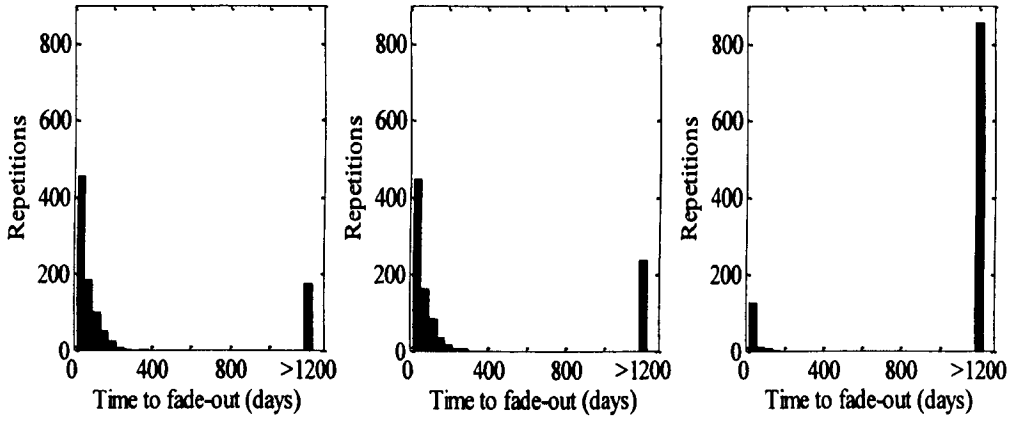


Figure 4.4 Time to fade-out of PRRSV for differences in the contact structure of the herd following introduction of one infectious gilt (herd size 327 sows)

Left: under normal conditions of the model (rate of transmission of 1 x 10^{-3} between pigs in different weaner, grower and finisher pens within a house), middle: rate of transmission of 0.1 between pigs in different weaner, grower and finisher pens within a house, right: 100 fold increase in the rate of transmission between all pigs in the herd compared with normal conditions of the model (as shown on the left)

4.3.4 The influence of herd size on persistence of PRRSV

The probability of persistence for >1200 days with herd sizes of 75, 150, 300 and 600 were 4%, 13.4%, 20.4% and 18.2% respectively. The median (range) time to fade-out for the herd sizes above was 44 (0-374), 39 (0-422), 40 (0-740) and 37 (0-897) days respectively.

4.3.5 The within-herd transmission dynamics of PRRSV following multiple introductions of virus

For a herd with 327 sows, an average of 147 breeding sows would be replaced each year. Given a probability of introducing infectious gilts into the herd via purchasing of 0.0025, 0.37 introductions of PRRSV would be expected annually. Similarly, assuming a probability of 0.04 infectious gilts, an average of 5.88 introductions of PRRSV would be expected annually. Virus was still present 1200 days after its first introduction in 32.4% of simulations when gilts had a 0.0025 probability of being infectious, compared with 17.6% of simulations when there was no re-introduction of virus (Figure 4.5). For probabilities of 0.005, 0.01, 0.02 and 0.04 of purchased gilts being infectious upon introduction, the probability of virus persisting at 1200 days were 43.0%, 57.3%, 78.9% and 90.4% in the models respectively (Figure 4.5).

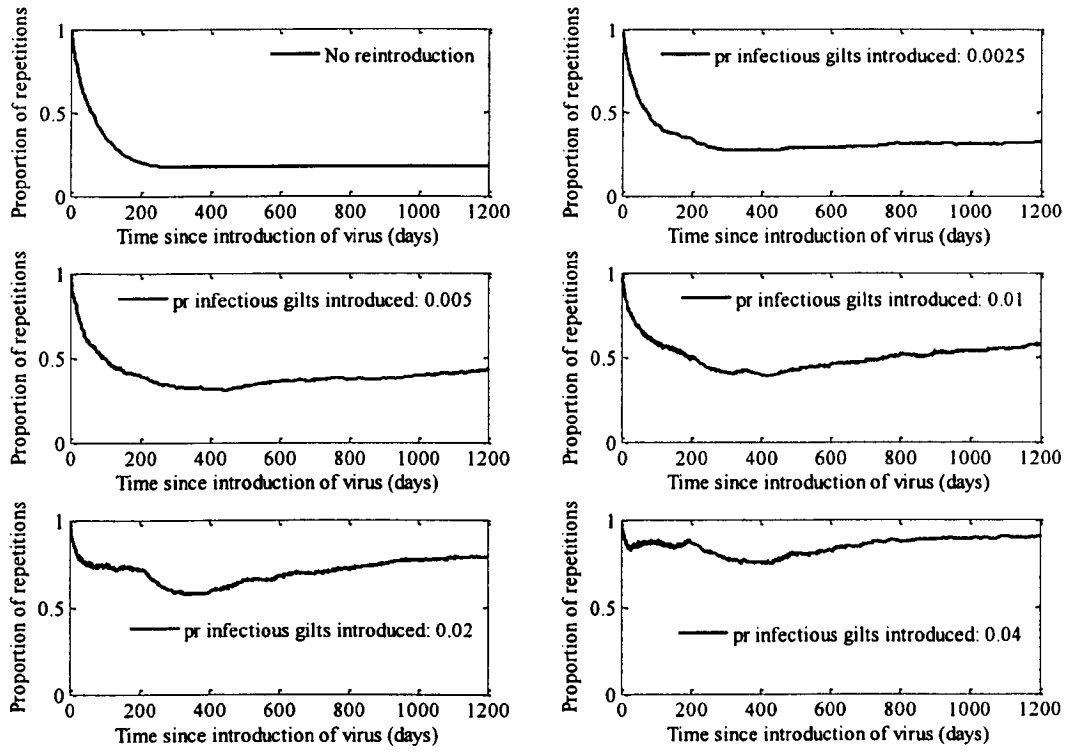


Figure 4.5 Proportion of simulations that were PRRSV positive by time for differences in the probability of replacement gilts being infectious upon introduction in to the simulated herd (herd size 327 sows)

4.4 Discussion

This Chapter presents a model of the within-herd transmission dynamics of PRRSV following single and multiple introductions and investigates fade-out and persistence. Cross-sectional serological field data were used to estimate the transmission parameters and the model was used to explain the different serological patterns observed. Since the data were used to specify only one parameter (β), this is effectively scaling rather than strictly fitting the model. The aim was to understand the serological patterns in the observed data, and to test the prediction that these patterns were consistent with fade-out and re-introduction of

PRRSV. A full sensitivity analysis of parameters was not conducted as this was not necessary for this purpose. In this Chapter demographic stochasticity (i.e. chance events in time and between individual pigs) is demonstrated to be an important determinant of PRRSV transmission dynamics.

The model included the age-structure of a pig herd and movements of pigs each week. For purposes of simplicity, non-routine movements of pigs because of returns to oestrus, abortions or decreased weight gain (rearing-pigs) were not modelled. Such movements are expected to influence the dynamics of virus within a herd. However, the patterns of the likely times to fade-out are not expected to change.

It was assumed that strains of PRRSV were homologous, with pigs immune to further infection whilst seropositive. If multiple antigenic types were a common feature of PRRSV so that immunity to previous infection is circumvented, then the estimated transmission parameter would be too high. It would not, however, change the overall conclusions. Indeed, the relatively low transmissibility of PRRSV and high risk of fade-out reported here suggest that co-circulation of antigenically different viral types is uncommon. Although there is considerable genetic variability between PRRSV isolates, its role in persistence of infection within herds is likely to be small.

The transmission parameter (β) was scaled from the data on the assumption that the observed seroprevalence in the field study herds were generated from the introduction of a single infectious gilt. The results have shown that multiple introductions are important in determining fade-out / persistence, and therefore serological patterns, so that it is possible that β was over-estimated in this study. This is an internal inconsistency that should be addressed in future work by also considering the transmission of PRRSV between herds. However, the conclusion (that fade-out and re-introduction are key processes) is more convincing if the observed data were created by re-introduction.

A high probability of fade-out of virus was documented in this theoretical study. This suggests a low transmissibility of PRRSV within a herd and a requirement for close contact to transmit PRRSV between pigs. On farms, transmission to sentinel pigs over 1 - 2.5 metres has been demonstrated (Wills *et al.*, 1997b, Otake *et al.*, 2002a), but attempts to transmit virus between buildings have led to conflicting results (Otake *et al.*, 2002a, Trincado *et al.*, 2004, Pitkin *et al.*, 2009). Another author has reported slow transmission of PRRSV in naturally infected commercial herds, even within individual pens of pigs (Houben *et al.*, 1995). Following single introduction, fade-out of virus was likely to occur within the breeding herd in the model but was unlikely once the virus spread to rearing-pigs. Another researcher has also reported that virus did not reach post-weaning pigs following introduction (Gordon, 1992).

Heterogeneities in serological profiles and differences in their likely state of transmission dynamics suggest that different herds are at different stages of viral introduction, persistence and fade-out. These differences will ultimately be influenced by the proportion of susceptible and exposed pigs in the herd. In herds with high levels of exposed, and therefore immune, pigs, the rate of contact between infectious and susceptible pigs is lower, making the herd less susceptible to outbreaks of clinical disease. Herds with large numbers of susceptible pigs are vulnerable to outbreaks of clinical PRRS (Dee and Joo, 1994b) following introduction of virus from a different group within the herd or from another herd via infectious replacement stock, aerosol (Pitkin *et al.*, 2009) or vectors such as insects or birds (Zimmermann *et al.*, 1997, Otake *et al.*, 2004).

Susceptible pigs can enter a herd through birth, replacement, or the decay of passive or active immunity. The rate of supply of susceptible pigs in to a herd is directly proportional to the herd size. The increased probability of persistence with increased herd size has been demonstrated in a previous mathematical model of PRRSV (Nodelijk *et al.*, 2000). In the current study, an increase in herd size was associated with a non-linear increase in the probability of persistence of virus. These results indicate that the decision to ensure high levels of herd immunity (by interventions such as vaccination) might be based on factors such as herd size, locality and the probability of re-introduction of virus.

The observed heterogeneity in serology between herds (Evans, *et al.*, 2008) raises the possibility that transmission of virus between herds is important in the persistence of PRRSV in a population of farms. If this is the case, then fade-out, introduction and re-introduction of virus are critical to persistence among herds. Whilst a single introduction of virus is the presentation of virus into a herd where it has previously been absent, re-introduction, such as through purchasing infectious stock is the presentation of virus into a herd where it has been, or still is, circulating.

Virus was still present at 1200 days in an additional 14.8% of simulations when 0.37 re-introductions per year were assumed compared with no re-introduction of virus. The likelihood of a re-introduction resulting in persistence of virus will be influenced by the availability of susceptible hosts in the recipient herd, previously discussed. The frequency of re-introduction influences the likelihood of virus persisting.

Isolating replacement stock in appropriate facilities could be an effective way of reducing the probability of re-introduction, reported previously (Edwards *et al.*, 1992, Evans *et al.*, 2008). Because transmission is more likely when contact between susceptible and infectious pigs is high, the segregation of pigs of different ages can reduce the spread of virus. Some authors have demonstrated elimination of PRRSV by only controlling the replacement breeding female population (Dee *et al.*, 1994c, Fano *et al.*, 2005). However, failure of elimination

in other studies by only partially segregating the rearing herd compared with all-in-all-out systems (Fano *et al.*, 2005) might suggest that whilst re-introduction could be an important source of persistence, the contact structure of the herd, particularly the rearing herd, might be important in maintaining virus.

4.5 Conclusions

Following a single introduction of PRRSV, fade-out of virus is likely to occur within the breeding group in the model: there is typically an insufficient supply of susceptibles into the adult herd (herd size 327 sows) to maintain transmission. Persistence within the simulated herd is more likely once PRRSV enters the farrowing house and so piglets and subsequently rearing-pigs become infected. Persistence is also more likely if gilts are not isolated from sows, as herd size increases (although this is non-linear), and as the proportion of infectious gilts introduced increases. Differences in the observed serological states between herds, combined with results from the mathematical model suggest that fade-out and re-introduction are not uncommon in pig herds and highlight the potential importance of transmission of virus between herds.

5 Chapter 5: The impact of control and elimination strategies for porcine reproductive and respiratory syndrome virus (PRRSV) on production

5.1 Introduction

Porcine reproductive and respiratory syndrome virus can be introduced in to a herd via replacement pigs (Edwards *et al.*, 1992), vectors (Zimmerman *et al.*, 1997, Otake *et al.*, 2004) and aerosol over short distances (Pitkin *et al.*, 2009). Isolating replacement gilts offsite for a period of time before introduction in to the main herd (Dee *et al.*, 1994c; Freese and Joo, 1994), introducing only known negative replacements (Dee *et al.*, 2000) and only using replacements that have been raised on-farm (Dee *et al.*, 1994c) have reduced the probability of introduction of PRRSV in case studies.

Following introduction of PRRSV, the type of control strategy used depends on the transmission of virus and clinical disease observed. Disease can be reduced in herds in which virus is transmitted slowly by reducing transmission between infectious and susceptible pigs further. In herds in which transmission of virus is high, control might involve maintaining high levels of endemicity, so that pigs become infected at a young age when disease is less costly.

Reducing transmission between susceptible and infectious pigs can be done by one or more of the following: reducing pathogen load, reducing the number or time that individuals are infectious or by reducing the number susceptible. The size of the susceptible population can be reduced by segregating age groups into separate sub-populations or by reducing the availability of susceptible pigs, e.g. by vaccination, depopulation (Dee *et al.*, 1993; Dee *et al.*, 2000), testing and removing seronegative pigs (Plomgaard *et al.*, 1998; Dee and Molitor, 1998b) or by deliberately exposing pigs in the herd to virus (Desrosiers *et al.*, 2002; Fano *et al.*, 2005). In Chapter 4 the importance of age segregation in reducing transmission of PRRS virus within breeding herds was demonstrated. This is also supported by evidence from case studies where the use of offsite rearing of grower pigs has reduced transmission between different ages of pig (Dee *et al.*, 1993). In populations with a large number of susceptible pigs, a reduction in the number infectious has been demonstrated through culling viraemic or seropositive individuals (Dee and Molitor, 1998b; Dee *et al.*, 2001; Dee *et al.*, 2001; Yang *et al.*, 2008) and by depopulating parts of the herd (Dee *et al.*, 1993; Dee and Joo, 1994a).

The elimination of an organism is its complete removal from a population where it has been present (Cockburn, 1963) and occurs when the basic reproduction number is less than one for a prolonged period of time (Anderson and May, 1992). Elimination may occur naturally by fade out or by physical intervention using some of the strategies mentioned above. For fade out, this is most likely to occur either early in an epidemic, when the number of infectious individuals is small or late in an epidemic when the number of susceptible individuals is small. It has been possible to eliminate PRRSV from some herds in the field (Dee *et al.*, 1993; Fano *et al.*, 2005), although failed attempts are likely to be less enthusiastically reported. In addition, an increase in the probability of fade-out in smaller herds has been reported and theoretically postulated for PRRSV (Nodelijk *et al.*, 2000; Chapter 5) and analysis of cross-sectional serological data suggests that fade-out of PRRSV might be a relatively common phenomenon (Chapter 5). This evidence suggests that PRRSV might not be difficult to eliminate. However, many herds become re-infected with virus (Dee *et al.*, 1997), highlighting the importance of determining sources of re-infection.

(Re-)introduction of virus can occur from within the herd or from outside. Within a herd, transmission is possible via nose-nose contact or via the movement of pigs, e.g. following replacement. Elimination of PRRSV from a pig herd involves a reduction in prevalence in all areas of the herd so that overall transmission is reduced. This might be possible by focusing on one group of pigs in the herd only

if this group is the source of persistence. Fade out of virus might occur in breeding sows in some herds but persistence may occur indefinitely once virus reaches rearing pigs (Chapter 4). This might suggest that elimination strategies may be better focused on the rearing herd. However, there have been conflicting reports in the field. Some authors have demonstrated elimination of PRRSV by controlling the replacement breeding female population only, despite virus being transmitted in the rearing herd (Dee *et al.*, 1994c).

Re-introduction of virus might cause outbreaks of clinical disease, the severity of which is influenced by the size of the susceptible population. The severity of clinical disease can be reduced by vaccination by making pigs immune. The ability of a vaccine to reduce clinical disease is influenced by the targeted age group(s), the efficacy of the vaccine and its effectiveness in a herd setting. It is likely that vaccine effectiveness may differ between herds because of differences in its storage, delivery and dosage but also because of differences in transmission dynamics of virus, both temporally and spatially.

In this Chapter a mathematical model framework is used to investigate the range of impacts of PRRSV on disease in a herd and to test strategies for control and elimination. These include depopulation of rearing pigs and vaccination of sows and gilts. Different efficacies of vaccine are considered and times to elimination compared. The herd structure, its demography and the model parameters are described in Chapter 4. The impact of infection on clinical disease was estimated

from the literature; production losses following PRRSV infection were compared with field outbreak reports.

5.2 Materials and methods

5.2.1 Model structure and epidemiological states

The structure and the demography assumptions within the mathematical model are described in Chapter 4. The demography assumptions are fixed with a gestation length of 115 days, a weaning age of four weeks and a slaughter age of 24 weeks. Gilts join the sow herd at 33 weeks of age and sows have a reproductive cycle of 21 weeks, which they complete approximately six times before being culled. Pigs within the simulated herd belong to one of the following mutually exclusive states: 1) passively immune (M); 2) susceptible (S); 3) infected (and infectious) (I); 4) recovered and seropositive (immune and no longer infectious) (R_{pos}); 5) recovered and seronegative (susceptible to reinfection) (R_{neg}). Parameters determining the natural history of infection are described in Chapter 4. In this study it was assumed that the rate of loss of immunity from natural infection was equal to the rate of loss of immunity induced by vaccination. The probability of a pig entering the vaccinated group following vaccination was dependent on the efficacy of the vaccine.

5.2.2 Impact of infection on clinical disease

Compared with Chapter 4, pigs can move or die following infection, as described below:

5.2.2.1 Sows

In the absence of virus, 12% and 3% of sows return to oestrus at 21 and 42 days post- service in the model respectively (Whittemore, 1993). The exact number was taken from a binomial distribution and sows were chosen at random. Sows that returned to oestrus joined the next batch of sows to be served and aged by one parity (thereby increasing their probability of progression through the herd and their eventual culling). The probability of infected sows returning was varied in the model and conception rates compared with outbreak data from herds from European strains of PRRS (Pejsak and Markowska-Daniel, 1997; Gordon, 1992).

Sows could abort or farrow prematurely in the model if they were infected after 42 days gestation (week 6). The probabilities were varied in the model and compared with outbreak data from European herds (Pejsak and Markowska-Daniel, 1997; Gordon, 1992). The cut-off between abortion and premature farrowing was defined at week 15; a sow that was infected post-week 14 was counted as a premature farrowing. It was assumed that all piglets born to sows that aborted or farrowed prematurely were dead at birth.

5.2.2.2 Replacement gilts

Replacement pigs were selected from the rearing herd each week and remained in the gilt house nine weeks before being served. At the time of selection from the rearing herd, the batch of sows that the gilts joined was 13 weeks through gestation. The number of gilts selected each week to join a particular batch was therefore dependent on the 'ideal' batch size (which allowed for some sows to be culled prior to service) and the number of sows already in that batch. To keep the herd size stable, movements of sows occurred only in the model up until week 13 of gestation; after this time the correct number of gilts would have been selected to join the relative batch. Sows returning or aborting after week 13 remained in the herd and moved through the cycle as normal.

5.2.2.3 Rearing pigs

Kranker *et al.*, (1998) inoculated sows at different stages of gestation with a European strain of PRRSV and determined the impact on piglets born alive, piglets born dead and piglets that survived pre-weaning. At 42-43 days gestation they observed 77.3% born alive, 13.6% mummified/stillborn and 9.1% dead pre-weaning, at 72 days, they observed 69.9% of piglets born alive, 14% mummified/stillborn and 16.3% of piglets dead pre-weaning and at 80-90 days gestation, they observed 43.9% born alive, 24.5% mummified/stillborn and 31.6% dead pre-weaning. In the model, these parameters are used for sows infected with PRRSV at 42-69 days, 70-79 days and 80-90 days gestation respectively. The

majority of weak-born piglets are observed to die in the first week of life in case reports of outbreaks (Gordon, 1992). All piglets that were assumed to die were therefore removed from the model at birth.

Baseline pre- and post-weaning mortality rates of 7% and 3% were assumed respectively (Whittemore, 1993). These are approximate estimates of mortality in pig herds in GB before PRRSV emerged. The probability that an infected pig died post-weaning was varied from 0.0 to 0.5 in the model and compared to percentage post-weaning mortality rates in case reports (Hopper *et al.*, 1992; Stevenson *et al.*, 1993; Neumann *et al.*, 2005). Pigs that died post-weaning were not removed from the model but calculated at slaughter.

From US studies, PRRSV seropositive pigs gained 18g less per day from 8-16 weeks and 22 g less per day from 16-24 weeks than did seronegative pigs of the same age (Regula *et al.*, 2000). In the model, the number of seropositive pigs within a batch was summed each day and the weight not gained per batch calculated. At slaughter, this gave the cumulative weight not gained for the entire batch.

5.2.3 Measurement of economic impact

The measures of production outputted from the model are listed in Table 5.1.

Piglets	Rearing pigs	Sows
Born alive	Number underweight	Returns to oestrus at 21/42 days gestation
Mummified/stillborn	Weight not gained per pig underweight	Abortions
Pre-weaning mortality	Post-weaning mortality	Premature farrowings
Weaned	Number finished	Empty days

Table 5.1 Measures of production outputted from the model for piglets, rearing pigs and sows

5.2.4 Control and elimination strategies examined using the model framework

Before virus was introduced, the model was run for 1000 weeks to ensure demographic equilibrium. One infectious gilt was introduced into the gilt house (within a batch) and all other pigs in the herd were assumed susceptible. The model was run for 1000 repetitions for 1200 days to follow transmission dynamics and disease over one complete life cycle of pigs in the herd. The total number of pigs weaned and finished, the number of empty days and the cumulative weight not gained for rearing pigs was recorded over the 1200 days and compared for each repetition. The effectiveness of each control or elimination strategy was

investigated for 100 simulations in which virus were present in rearing pigs and sows. The outputs were compared for herd sizes of 100 and 400 sows.

5.2.4.1 Natural fade out of virus without intervention

The probability of natural fade out was observed without intervention for both herd sizes. To verify whether virus could persist independently in sows or rearing pigs following virus presence in young stock, all sows or all rearing pigs were assumed immune and the consequential impact on persistence of virus determined. Persistence of virus in both sows and rearing pigs for each intervention are presented.

5.2.4.2 Depopulation of the rearing herd

The rearing herd was depopulated for two or six weeks following either an increase of 20% of finished pigs underweight at slaughter or a decrease of 20% of piglets born alive. In each case, probabilities of persistence of virus and re-infection of rearing pigs were determined. During depopulation, it was assumed that negative replacement gilts were sourced until the newly-stocked rearing herd had replacements old enough to be selected.

5.2.4.3 Vaccination of sows and gilts

The probability of elimination of virus was determined when sows and gilts were vaccinated on one occasion. The ability of vaccine in reducing spread to rearing pigs was determined. Vaccination was implemented following either an increase of 20% of finished pigs underweight at slaughter or a decrease of 20% of piglets born alive.

5.2.4.4 Depopulation of the rearing herd for 6 weeks and vaccination of all sows and gilts

Combined depopulation of the rearing herd and vaccination of all sows and gilts was implemented in the simulated herd. The probability of elimination of PRRSV was compared when sows and gilts were vaccinated on one occasion only and when vaccination was used prior to every service. Based on times to elimination of PRRSV, extra pigs weaned and finished and fewer empty days and weight not gained were determined and compared with not implementing any control strategy. Probabilities of elimination were compared when virus was re-introduced at rates of 1/400 and 1/25 infectious gilts.

5.3 Results

5.3.1 Impact of PRRSV infection on production

Following the introduction of an infectious gilt, the first signs of infection were usually observed in sows, followed by piglets and then rearing pigs (Figures 5.1 – 5.3). The percentage of sows returning at either 21 or 42 days after service almost doubled immediately after introduction of virus in many simulations, sometimes increasing to 30-40% (Figure 5.1). In one simulation, 72.7% of sows within a batch returned to oestrus at 21 or 42 days, 124 weeks after introduction of virus. The number of abortions observed each week ranged from 0 - 10. Premature farrowings increased from a baseline of zero to 25-35% and in one herd was 50% of the total number of sows farrowing (Figure 5.1). Returns to oestrus, abortions and premature farrowings all contributed to an increase in the number of empty days, which increased by almost 300% for many simulations (Figure 5.1).

The number of piglets born alive per sow varied from 4.3 - 11.3. In one simulation, 4.3 piglets were born alive per sow 42 weeks after introduction of virus, with 64.2% of piglets mummified/stillborn and 7% of piglets dying pre-weaning. The largest decrease in number of piglets born alive per sow was observed on average between 20 and 50 weeks after introduction of virus (Figure 5.2).

The time to infection of the rearing herd (pigs of 5-24 weeks of age) varied from 1 - 128 weeks (mean 52 weeks). Infection of the rearing herd was associated with a sharp increase in post-weaning mortality from 3.3% to 8.5% (Figure 5.3), with over 80% of rearing pigs exposed to virus during their time in the herd. The average age of infection was approximately 13-14 weeks of age and pigs that were underweight at slaughter (those that had been exposed) were over 2g lighter than pigs that had not been exposed to virus (Figure 5.3).

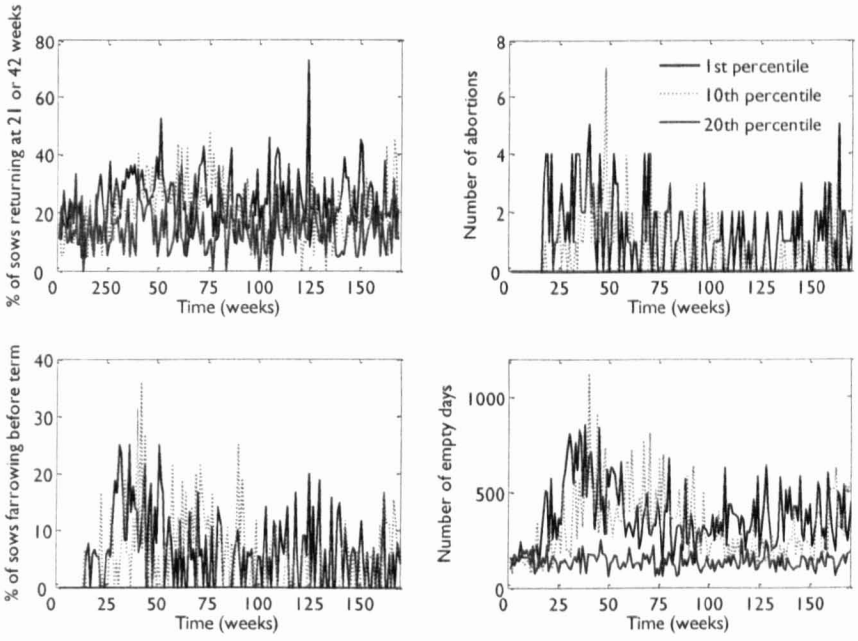


Figure 5.1 Production losses in sows as a result of PRRSV infection.

Lines indicate the 1st, 10th and 20th percentile based on the number of piglets born alive over 1200 days (1000 repetitions in total).

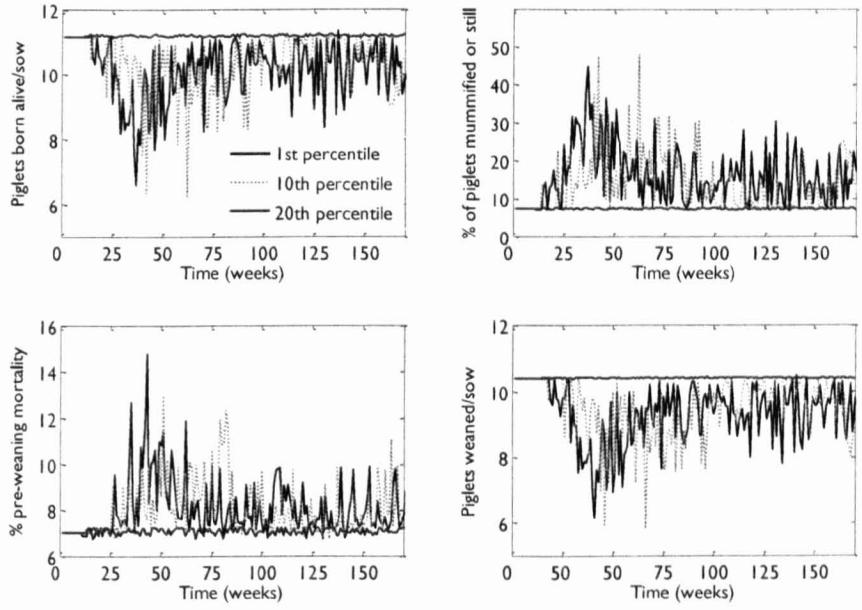


Figure 5.2 Production losses in piglets as a result of PRRSV infection.

Lines indicate the 1st, 10th and 20th percentile based on the number of piglets born alive over 1200 days (1000 repetitions in total).

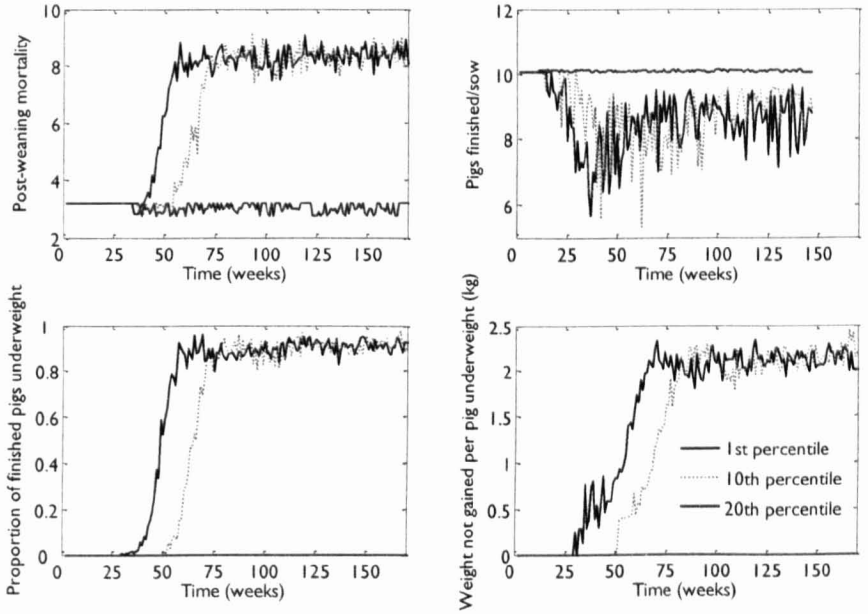


Figure 5.3 Production losses in rearing pigs as a result of PRRSV infection.

Lines indicate the 1st, 10th and 20th percentile based on the number of piglets born alive over 1200 days (1000 repetitions in total).

The impact of PRRSV infection on production was highly variable, both between and within simulations of 1200 days. Based on the total number of pigs finished over 1200 days, individual simulations from the 1st, 10th and 20th percentiles (of 1000 simulations) are presented for a 400 sow herd (Figures 5.1-5.3). The distribution is skewed, so that the median typically falls just below the 20th percentile. Compared with averages taken from 1000 simulations without virus, one simulation from the 20th percentile had 0.53% fewer piglets weaned and 0.62% fewer pigs finished over 1200 days (Table 5.2). Similarly, one simulation from the first percentile had 15% fewer piglets weaned, 16.1% fewer pigs finished, 172.9% more empty days and 28.5kg of weight not gained by finished pigs (Table 5.2).

Percentile	Number weaned	Number finished	Total number empty days	Total weight not gained (kg)
1st	24955 (15% ↓)	24199 (16.11% ↓)	64786 (172.86% ↑)	28.54
10th	26057 (11.26% ↓)	25248 (12.48% ↓)	55034 (131.79% ↑)	26.20
20th	29207 (0.53% ↓)	28668 (0.62% ↓)	23641 (0.43% ↓)	0
No virus	**29361.73**	**28846.94**	**23743.17**	**0**

Table 5.2 Production indicators over 1200 days for the 1st, 10th and 20th percentiles of 1000 simulations based on the total number of piglets born alive, compared with averages taken from 1000 simulations in the absence of virus.

Numbers in brackets indicate percentage increase or decrease in numbers over 1200 days, compared with no virus. Arrows indicate the direction of the increase or decrease.

5.3.2 Natural fade-out of virus without intervention

For herd sizes of 100 and 400 sows, natural fade-out of virus occurred in 55/100 and 1/100 repetitions without intervention respectively, despite virus being present in rearing pigs (Figure 5.4). For both herd sizes, virus persisted in sows and in rearing pigs independently following infection of rearing pigs. When rearing pigs or sows were assumed seropositive for a herd size of 400 sows, virus was still present in sows in 84/100 simulations and in rearing pigs in 77/100 simulations at 1200 days.

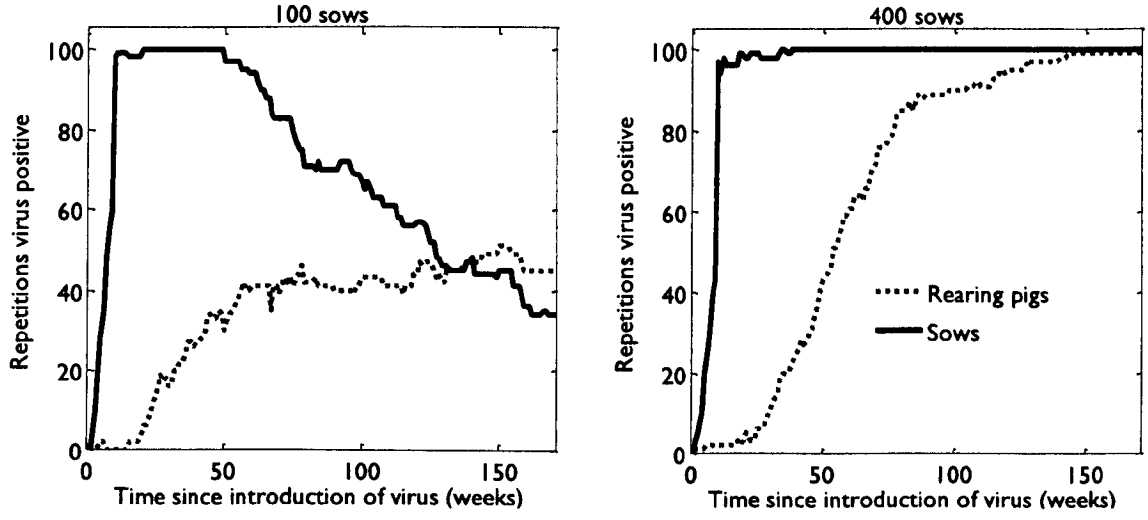


Figure 5.4 Number of repetitions virus positive by time (out of 100) for herd sizes of 100 and 400 if virus reaches rearing pigs (no intervention)

5.3.3 Depopulation of the rearing herd

Following an increase of 20% of finished pigs underweight at slaughter and depopulation of the rearing herd for 2 weeks, the number of simulations with virus at 1200 days for herd sizes of 100 or 400 sows did not decrease (Figure 5.5). For a herd size of 400 sows, re-infection of rearing pigs occurred within five weeks in 56/100 simulations and within 25, 50 and 75 weeks in 76/100, 10/100 and 12/100 simulations respectively (Figure 5.6). Depopulating the rearing herd for six consecutive weeks did reduce the proportion of herds in which rearing pigs were positive: for a 100 sow herd this was a decrease from 55/100 to 36/100 simulations with infected rearing pigs.

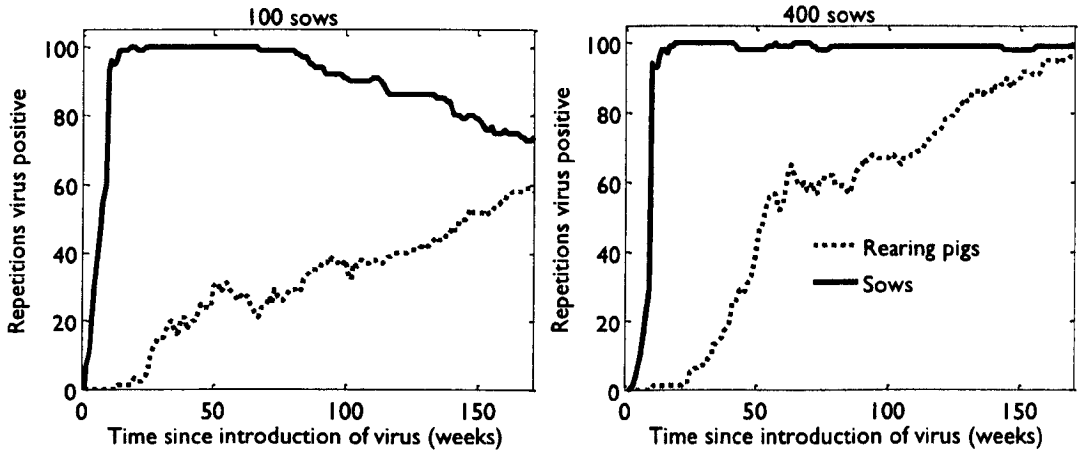


Figure 5.5 Number of repetitions virus positive by time (out of 100) for herd sizes of 100 and 400 if virus reached rearing pigs (Intervention: Depopulation of rearing pigs for two consecutive weeks)

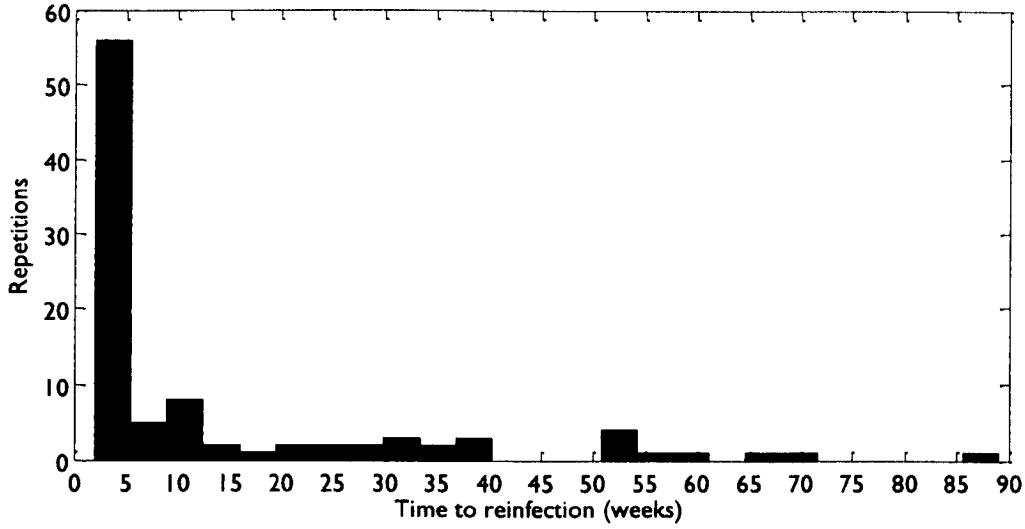


Figure 5.6 Time to re-infection following depopulation of the rearing herd of a herd size of 400 sows for two consecutive weeks

5.3.4 Vaccination of sows and gilts

Vaccination of sows and gilts with a 60% or 100% efficacious vaccine when there was a decrease in piglets born alive of 20% prevented virus reaching rearing pigs in 3.5% and 5.4% of simulations respectively, compared with vaccinating when there was an increase in pigs underweight of 20%. For herds where virus reached the rearing pigs, vaccination of sows and gilts with a 60% efficacious vaccine when there was a decrease of piglets born alive of 20% resulted in 3.4% more piglets born alive over 1200 days, 23.4% less empty days, 51.8% fewer abortions, 59.2% fewer returns to oestrus, 4.2% fewer pigs underweight at slaughter and 3.5% more pigs finished, compared with vaccinating when there was an increase of pigs underweight of 20% (Table 5.3).

For herds where virus reached the rearing pigs, vaccination with a 60% efficacious vaccine when there was an increase of pigs underweight of 20% resulted in 5.45% more piglets born alive over 1200 days, 23.82% fewer empty days, 40.34% fewer abortions and 40.66% fewer returns to oestrus, compared with no intervention (Table 5.3). Vaccination was associated with a decrease of 14.8% pigs underweight at slaughter and 5.6% more pigs finished.

	Piglets born	**Empty days**	**Abortions**	**Returns to oestrus**	**Pigs underweight at slaughter**	**Pigs finished**
No intervention	28024.8	57737.0	166.4	271.8	10524.6	25141.7
Vaccination when pigs underweight at slaughter >20%	29551.5	43981.6	99.3	161.3	12079.5	26541.6
Vaccination when piglets born alive per sow <20%	30548.1	33691.9	47.9	65.8	11570.7	27471.2

Table 5.3 Production losses if virus reached rearing pigs for no intervention and for vaccination of sows and gilts with a 60% efficacious vaccine before service when piglets born alive <20% or pigs underweight at slaughter >20%

5.3.5 Depopulation of the rearing herd for six weeks and vaccination of all sows and gilts

Depopulation of the rearing herd for six weeks and vaccination of sows and gilts at one time point eliminated virus in some simulations. For a 400 sow herd and

assuming vaccine efficacies of 20%, 40%, 60%, 80% and 100%, virus was eliminated from rearing pigs within 1200 days in 19%, 28%, 33%, 33% and 79% of simulations by 1200 days respectively (Figure 5.7). For sows, virus was eliminated in 0%, 0%, 1%, 6% and 64% of simulations respectively.

5.3.6 Impact of elimination on total pigs finished

The probability of successful elimination increased following subsequent vaccination of all sows and gilts before every service (Figure 5.7). For vaccine efficacies of 20%, 40%, 60%, 80% and 100%, subsequent vaccination increased the percentage of simulations without virus in rearing pigs by 4%, 6%, 16%, 43% and 16% and in sows by 3%, 15%, 39%, 71% and 30% respectively (Figure 5.7). For a vaccine efficacy of 100%, 5% of simulations had viraemic rearing pigs at 1200 days and 6% had viraemic sows.

Vaccination with a higher efficacy eliminated virus earlier compared with vaccination with lower efficacy (Figure 5.8). On average, 3710 more pigs were finished over 1200 days in 1000 simulations without virus (for a 400 sow herd), compared with 100 simulations with virus present in sows and rearing pigs (Figure 5.8). Elimination of virus in weeks 50, 75, 100, 125 and 175 was associated with an increase the number of pigs finished by 3664.6, 2933.3, 1902.9, 1149.4 and 531.1 respectively. The average time to elimination of virus for vaccine efficacies of 20%, 40%, 60%, 80% and 100% were 90.7, 123.8, 129.7,

114.4 and 71.8 weeks, corresponding to an increase in the number of pigs finished by 2259, 1173, 1011, 1431 and 3061 respectively for those simulations in which virus was eliminated. Only with vaccine efficacies of > 80% was virus eliminated within the first 50 weeks. Using a vaccine efficacy of 100%, 20/93 simulations in which virus were eliminated occurred within 50 weeks after introduction of virus (Figure 5.8).

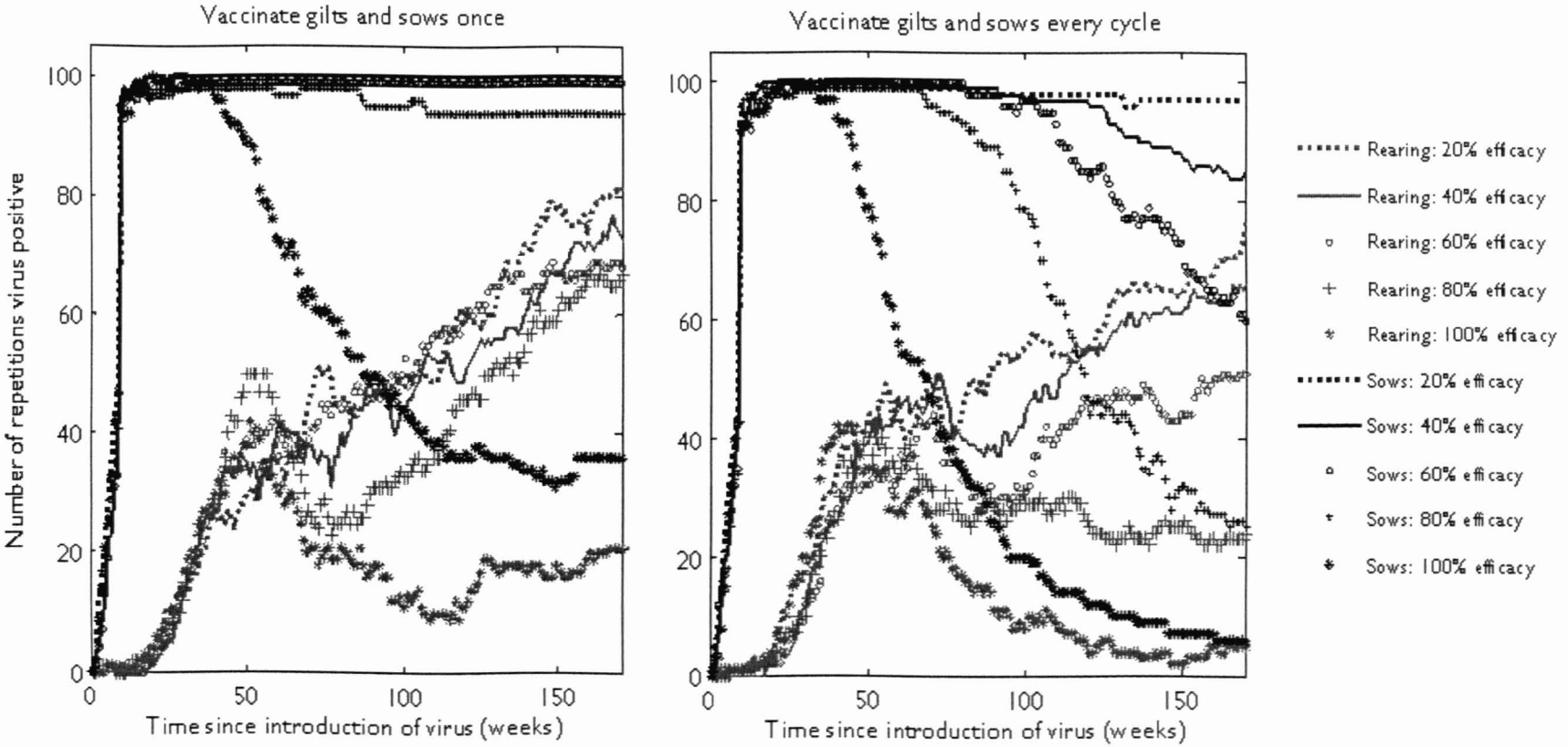
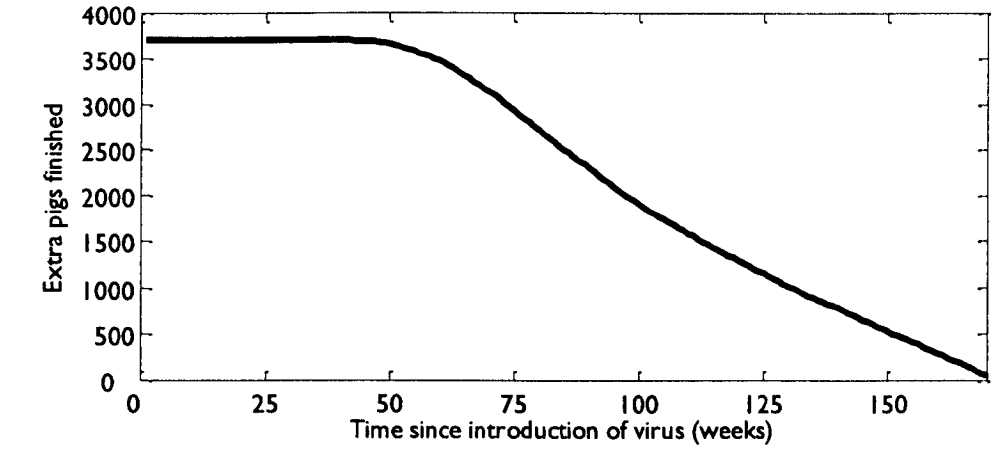


Figure 5.7 Number of repetitions virus positive by time (out of 100) if virus reaches rearing pigs (intervention: depopulation of the rearing herd for 6 consecutive weeks and vaccination of gilts and sows at one time point (left) or at one time point and every cycle thereafter (right))



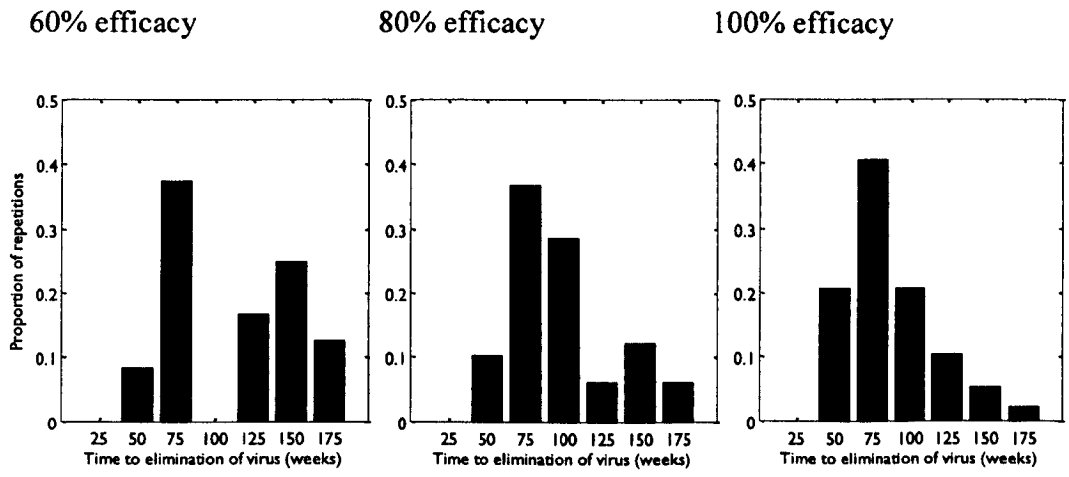


Figure 5.8 Extra pigs that would be finished over 1200 days given different times to elimination of virus (top). Time to elimination of virus for different efficacies of vaccine when the rearing herd is depopulated for 6 weeks and vaccination is used immediately and at every service interval thereafter (bottom)

5.3.7 Impact of re-introduction on success of virus elimination

Re-introduction of virus lowered the probability of successful elimination for a 400 sow herd (Figure 5.9). For vaccine efficacies of 20%, 40% and 80%, virus was present in an additional 3/100, 7/100 and 3/100 simulations when virus was re-introduced at a rate of 1/400 compared with no re-introduction of virus. For efficacies of 60% and 100%, 7/100 more simulations did not have virus at 1200 days and for an efficacy of 100%, there was no change. For efficacies of 20%, 40%, 60%, 80% and 100%, virus was not eliminated in an additional 61/100, 54/100, 28/100, 10/100 and 3/100 simulations respectively when virus was re-introduced at a rate of 1/25, compared with no re-introduction of virus.

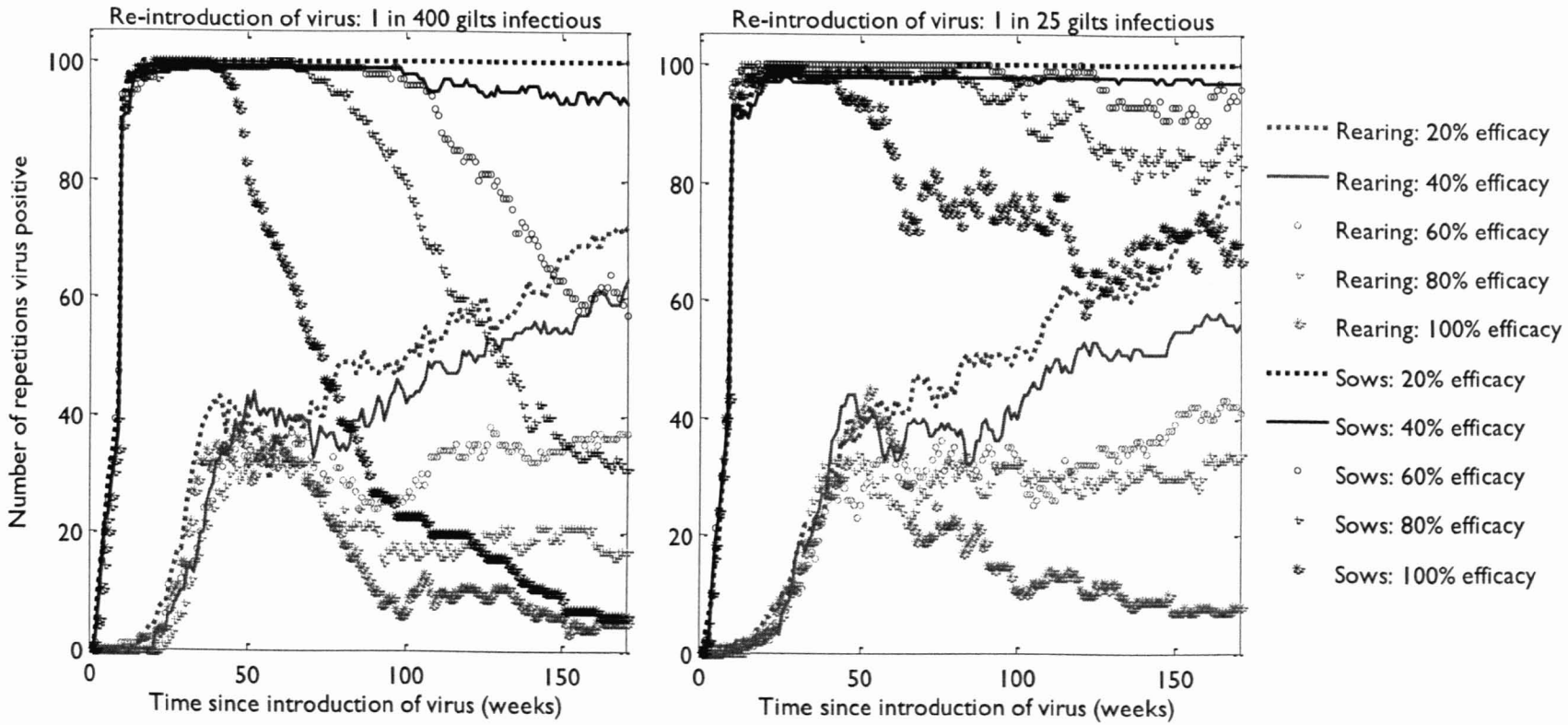


Figure 5.9 Number of repetitions virus positive by time (out of 100) following depopulation of the rearing herd for 6 consecutive weeks and blanket vaccination of gilts and sows followed by vaccination at every cycle thereafter for different rates of re-introduction of virus

5.4 Discussion

This research presents a stochastic model of the transmission dynamics of PRRSV in a simulated pig herd, its impact on production, and mitigation of this with different interventions. The model demography and assumptions have already been discussed (Chapter 4); parameters came from the literature and the transmission parameter was estimated from existing cross sectional field serology data for 50 pigs from each of 103 pig herds. Impacts of infection with PRRSV on production were estimated from the literature and from field studies of PRRSV outbreaks; European data were used for these estimates, where possible.

Production losses recorded in this study aimed to provide a detailed account of the impact of infection on a range of parameters in all areas of the herd, including piglets, rearing pigs and sows. This provided a time-dependent account of the impact of virus within different age groups concurrently and enabled an estimation of the impact of different interventions on a range of production parameters. Estimates of infection of sows on the viability of their piglets at birth were taken from a study that infected sows at different stages of gestation with a European strain of the virus (Kranker *et al.*, 1998). However, numbers of piglets born alive, mummified and those that died pre-weaning agreed with field accounts of PRRSV infection, which report the number of piglets born alive to drop from 12.4 to 7.5 (Hopper *et al.*, 1992), piglets born mummified/stillborn to reach 18.8% (Hopper *et al.*, 1992) and pre-weaning mortality to vary between 8.10% over a year (Pejsak and Markowska-Daniel, 1997) to 10% (Gordon, 1992) to 30-60%

over the worst four weeks of an outbreak (Gordon, 1992). In sows, authors have reported returns to oestrus and premature farrowing to reach 50% and 76% respectively (Gordon, 1992), which are within the range described here.

Lastly, for rearing pigs, post-weaning mortality has varied from 1 - 7.4% (Hopper *et al.*, 1992; Stevenson *et al.*, 1993; Neumann *et al.*, 2005) and following depopulation of rearing pigs in US herds, post-weaning mortality has decreased by 6-11% (Dee *et al.*, 1997). Post-weaning mortality rates of 5-6% was assumed, which is within these estimates. A decrease in weight of 18g and 22g for seropositive pigs from 8-16 weeks of age and 16-24 weeks of age were assumed respectively (Regula *et al.*, 2000). From the model outputs, approximately 90% of rearing pigs were exposed to virus within the herd by 24 weeks and given an average age of infection of 13-14 weeks of age pigs that had been exposed to virus were on average 2.0-2.4kg lighter than pigs that had not been exposed. Observations by Hopper (1992) have shown average dead weight to fall by 3kg in bacon pigs and observed an increase of 13% of pigs underweight. Differences in the percentage of pigs underweight reported here may result from the model not allowing for compensatory growth. It is possible that for pigs in infected herds, the impact of infection on weight not gained is masked by increased food rations and ad-lib feeding. Further studies would need to quantify this.

The impact of PRRSV infection on production was highly variable, both between and within individual simulations. Significant between-herd variability in the

severity of PRRS, the duration of clinical signs and the economic impact has been reported in field studies (Goldberg *et al.*, 2000, Baysinger *et al.*, 1997, Pejsak and Markowska-Daniel, 1997, Pejsak *et al.*, 1997). Cycles of clinical disease have also been observed within individual herds (Stevenson *et al.*, 1993; Gordon, 1992; Dee *et al.*, 1994b). This observed variability could result from waning protective immunity, which lasts between 4.5 - 20 months (Yoon *et al.*, 1995, Desrosiers *et al.*, 2002), different strains of circulating virus or differences in the transmission dynamics of virus in time and space. Some authors have investigated factors associated with the variability in clinical signs of infection and pig antibody levels between herds. These have observed that the purchasing and isolation of replacement stock, pig density in the region and number of pigs in the herd to be associated with up to 50% of the between-herd variability (Baysinger *et al.*, 1997; Evans *et al.*, 2008). These factors are related to virus introduction (i.e. purchasing, isolation) and persistence (i.e. numbers of pigs). In this study, a single introduction of virus and a constant herd size was assumed, so that any differences observed between simulations were generated from chance events in the transmission dynamics of virus. These differences at any time are important in determining the success of a particular intervention strategy for PRRSV because these will determine the presence of infectious and susceptible individuals in time and space. In particular, the presence of infectious and susceptible pigs in the farrowing house determines the success of a particular intervention strategy because it determines the probability of transmission between different age groups within the herd; from infectious sows to piglets or from infectious piglets to sows. The presence of infectious sows within the farrowing house determined the time

to re-infection of newly weaned rearing pigs following depopulation in the model, which varied from 2-86 weeks for a 400 sow herd. In over 50% of simulations elimination occurred within the first five weeks. The source of re-infection was likely to be infectious sows and 1 - 2 week old piglets in the farrowing house. These piglets would have been born before the rearing herd was depopulated (pigs of 5-24 weeks of age) and transmitted virus to rearing pigs after weaning 2 - 3 weeks later. The success of elimination by depopulation is therefore dependent on the absence of viraemic sows and a lower weaning age, so that piglets are less likely to become infected before weaning.

Results from the model show that rearing pigs can remain free of virus for a long time, despite virus being present in sows. Other authors have reported re-infection of rearing pigs up to 16 months after elimination (Dee *et al.*, 1994a), which the authors attributed to aerosol transmission or a carrier pig. Results here suggest that many instances of re-infection might arise from within the herd and not necessarily from outside. Ways of reducing re-infection following depopulation may involve vaccinating sows and gilts concurrently; the independent persistence of virus in both sows and rearing pigs presented here highlights the need for elimination strategies to target both populations. This procedure has also been reported to be successful in another study (Dee *et al.*, 1998a).

The ability of a vaccine in preventing transmission of virus within a herd is determined by both its efficacy and effectiveness. Whilst the efficacy of a vaccine

is its ability to elicit an appropriate antibody response in an individual pig, the effectiveness is its ability to reduce transmission within a population. The latter is influenced by the strain of the virus and the ability of the vaccine to confer the same homologous protection, the storage and administration of the vaccine and the state of the transmission dynamics of virus. In the model, vaccination with 100% efficacy combined with depopulation of the rearing herd for six weeks did not eliminate virus in 5% of simulations, even when vaccination was used prior to every service. These results highlight the importance of the transmission dynamics at the time of the implementation. Despite this variability, vaccination reduced the probability of rearing pigs becoming infectious in the model (by 3.5% for a vaccine with 60% efficacy) and was associated with a significant reduction in clinical disease, especially for sows, following its implementation. Despite these observations, the rate of re-introduction of virus in the field will influence the effectiveness of vaccination in reducing clinical disease and eliminating virus. For example, for vaccine efficacies of 80% and 100%, a rate of re-introduction of 1/400 did not influence the probability of elimination but a rate of 1/25 reduced the probability of successful elimination by 54% and 61% respectively.

5.5 Conclusions

The success of any control or elimination strategy for PRRSV is dependent on the transmission dynamics of virus in time and space at the time of implementation: failure in this study was usually caused by presence of infectious pigs within the farrowing house and transmission to other age groups. Persistence of PRRSV

within sows and rearing pigs occurs independently and consequently, elimination strategies that target both populations are required. Elimination is most likely when sows are vaccinated and the rearing herd is depopulated for long enough to prevent infectious piglets entering weaning accommodation. Vaccination can prevent virus from being transmitted to rearing pigs if implemented early during an epidemic and can reduce clinical disease within the herd, especially in sows.

6 Discussion and conclusions

6.1 Introduction

At the start of this thesis I introduced respiratory diseases in pigs and highlighted their importance in reducing pig health. These diseases also impact on the productivity and sustainability of pig herds. The aim of this thesis was to investigate the associations between respiratory pathogens and morbidity and mortality in pig herds in GB. This was addressed in Chapters 2 - 5, as follows:

- Chapter 2: an investigation of the prevalence and incidence of common respiratory diseases in GB and their association with one another and with post-weaning mortality. The key findings were that pathogens clustered on farms, suggesting similar risk factors for infection or persistence on a farm and that there was a non-linear positive association between number of pathogens on a farm and post-weaning mortality.

- Chapter 3: an investigation of herd cross-sectional serology data for PRRSV and the determination of management factors associated with within and between-herd variability in pig antibodies. The key finding was that factors associated with herd infection include the proximity to other pig herds, having

>250 sows, not isolating purchased stock and not isolating for sufficiently long, so that pigs were still infectious when they entered the herd.

- Chapter 4: the development of a mathematical model of a pig farm and the investigation of mechanisms for persistence and fade-out of PRRSV. The key findings were that there was a high frequency of fade out in breeding pigs before virus reached young stock and that there was an increased probability of persistence of virus in the following situations: in young stock, large herds, herds with increased contact between age groups and herds where there was frequent re-introduction of virus.

- Chapter 5: a summary of the range of impacts of PRRSV on disease in a herd and the impact of various control and elimination strategies. The key findings were that PRRSV was difficult to eliminate without targeting both rearing pigs and sows. Rapid vaccination of sows once there was an increase in preweaning still births could be used to reduce the spread of virus to rearing pigs.

Below I discuss the implications of this research and how it has changed our understanding of respiratory disease in pigs and suggestions for further research.

6.2 Research findings and implications

PRRSV is a pig specific disease with previous research that indicates that it is transmitted reasonably slowly. The age-related antibody profiles of PRRSV were highly heterogeneous between farms (Chapter 3) and mathematical modelling

indicated that this large variability could be explained by the time since introduction of virus (Chapter 4). The mathematical models indicate that this does mean that in certain situations the virus can die out without intervention. This supports the previous report of natural fade out of PRRSV in one pig herd in the Netherlands (Nodelijk *et al.*, 2000). This thesis is the first study that has provided evidence for the biological basis of fade out.

The apparent within and between herd differences of the impact of PRRSV on performance and disease have previously been attributed to different antigenic types and therefore virulence and / or transmissibility. This thesis provides evidence that the transmission dynamics of PRRSV might account for some of the differences observed within a herd over time and also between herds. Further studies are required to determine whether antigenic types differ in virulence, transmissibility or ability to elicit an effective immune response in the pig. Results from such studies would inform on assumptions made on the homogeneity or disease from PRRSV assumed in the current research.

Fade out is most likely in small, isolated herds that breed their own replacements or practice good isolation of incoming stock. These herds are less vulnerable to re-introduction of PRRSV because of their situation. If re-introduction occurs in herds with these characteristics an erratic clinical presentation might be observed such as irregular epidemics of small litter sizes, increased dead piglets and weak live piglets. These farmers and their vets might think that the herd is persistently

infected. If re-introduction is a risk then breeding gilts and sows could be tested regularly to provide a means of early detection of PRRSV so that appropriate measures, e.g. isolation or culling of infectious stock, could be taken before virus persists. Alternatively, vaccination could be used to reduce the probability of virus reaching rearing pigs; but only if implemented early (Chapter 5). If virus reaches the farrowing house and rearing pigs, it is more likely to persist within the herd (Chapter 4). Persistence and re-introduction of PRRSV are likely in large herds in close proximity with poor biosecurity for incoming stock. Consequently, herd size and location could be used by the pig industry in England to determine whether an elimination or control programme is most appropriate. This is a move away from the idea that the pig industry has one strategy for all herds. In the west of England it might be possible to eliminate PRRSV but in pig dense areas of the east of England control is most likely to lead to a stable state.

If national elimination is the desired homogenous approach, the west of England region and other such low density pig regions could provide starting locations for elimination schemes because of the lower risk of localised transmission to neighbouring herds. The difficulty in eliminating PRRSV even with targeting both rearing pig and sows should not be underestimated because of the high probability of transmission occurring in the farrowing house (Chapter 5). Virus can remain present in sows but not in the rearing herd for up to 86 weeks after depopulation of rearing pigs. Depending on the probability of (re)-introduction of virus, elimination might therefore still be worthwhile, but only if the cumulative impact on health was less over this period than if elimination had not been attempted.

Results from this thesis have implications for the future development of the pig industry in the UK and highlights the possibility that several smaller herds in pig sparse regions might have less disease, compared with larger herds situated in pig dense regions.

Effective control of a pathogen involves reducing transmission between susceptible and infectious individuals so that clinical disease is reduced. This is important in improving animal welfare on the farm but also improves staff morale, production efficiency and for bacterial infections reduces the use of antibiotics. The control of PRRSV is important in improving farrowing rates and therefore management efficiency because of less time spent moving sows to be re-serviced or culled. It also optimises the number of sows that farrow and reduces pre-weaning mortality, increasing the number of piglets weaned, so that less time is spent cross-fostering piglets and weaning sows at different times. It also reduces problems associated with feeding regimes, which can often be disrupted during an epidemic because of the requirement to feed pigs more in order to produce an ideal slaughter weight and to avoid being penalized at the abattoir.

Considering the probability of fade out for PRRSV, it might be unreasonable to assume that all respiratory pathogens were still present on farms based on clinical history (Chapter 2). However; the nature of a pathogen is a key feature for persistence or fade out. Factors likely to lead to persistence are long duration of infectiousness and/ or ability to survive outside the host in the environment or

other reservoir hosts. The pathogens studied in Chapter 2 were viruses, bacteria and a syndrome, all with different periods of infectiousness and reservoirs. Given the lack of clarity of results from Chapter 2, more than veterinary recall is required to make a good study of multiple infections.

The presence of respiratory disease in a pig herd is a consequence of the metapopulation dynamics of multiple pathogens, i.e. their transmission within and between populations of pig herds. Given the results from Chapter 2 it appears that pathogens causing respiratory disease were clustered on individual farms. Factors that influence the presence of pathogens in a herd include route of introduction and methods for persistence. This might suggest that it is possible to control many pathogens using a common strategy. A control effort, if made, could be targeted at several pathogens with similar characteristics.

The elimination of one pathogen from a herd might not significantly reduce clinical disease. This comes from the observation that infection with a larger number of pathogens was associated with a non-linear increase in post-weaning mortality (Chapter 2), i.e. the size of the incremental increases in mortality gradually lessened, the more pathogens herds were infected with. For a pathogen that has naturally faded out or that has been eliminated, the severity of disease will depend on the time to re-introduction and the number of susceptible pigs present (Chapter 4). Pathogens are therefore more easily identified and controlled when first introduced and when disease is high. Construction of a metapopulation

model that included many pathogens simultaneously might provide insight into the clustering of pathogens within herds, and the consequent patterns of clinical disease and lost production.

The mathematical model represented a pig herd in its age structure, contact structure, management and demography. The model was important in understanding the serological profiles from the field data and the processes involved in fade out and persistence of PRRSV. It was also a useful way of comparing different control and elimination strategies, whilst controlling for factors that would influence dynamics in actual herds. These include time of virus introduction, movements of pigs on and off the farm and culling rates. Some assumptions of the model were made in order to simplify some processes within pig herds. Some of these are discussed in Chapters 4-5 and include non-routine movements of pigs, indoor pig production and weekly (not batch) farrowing. At the time of data collection, most of the herds were farrowing sows on a weekly basis. However, since the implementation of this study in 2003 / 2004, there has been a change in the way pigs are produced within herds, with many producers now farrowing a batch of sows every three weeks. The model could be further developed to explore transmission dynamics within a batch system and also to extend it to a production style more consistent with an outdoor herd. The relative probability of transmission between herds is required in order to further develop these models for practical use by the industry. Such studies might indicate whether the value of the transmission parameter, β, was reasonable in Chapters 4

and 5. It is possible that β was a mean value of the within and between herd transmission parameters.

6.3 Conclusions

This thesis has contributed to our understanding of respiratory disease, particularly PRRSV, in the GB pig population. The thesis considered multiple interacting factors that are associated with herd infection, including the role of management, characteristics of the herd, presence of multiple pathogens and control and elimination strategies. The clustering of pathogens on individual farms highlighted common risk factors for herd infection or persistence. For PRRSV, these included the proximity of herds to other pig herds, having >250 sows, not isolating purchased stock and not isolating for sufficiently long enough. With the use of statistical and mathematical models this thesis provides evidence for the biological basis and feasibility of fade out and re-introduction of PRRSV in individual farms and its association with apparent erratic behaviour. Mathematical models were also used to test strategies for PRRSV control and elimination. Results highlighted that in areas of GB where the density of pigs is low it might be possible to control PRRSV through elimination, whilst in larger herds in pig dense regions elimination might be difficult to achieve and control might give more stability. Significant improvements in production might not be observed unless several respiratory pathogens are eliminated from a herd and the long-term benefits will ultimately depend on the risk of (re)-introduction from both within and outside the herd.

References

Albina, E., Madec, F., Cariolet, R. and Torrison, J. (1994). Immune response and persistence of the porcine reproductive and respiratory syndrome virus in infected pigs and farm units. *The Veterinary Record* **134**, 567-573.

Albina, E., Piriou, L., Hutet, E., Cariolet, R. and L'Hospitalier, R. (1998). Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). *Veterinary Immunology and Immunopathology* **61**, 49-66.

Alexopoulos, C., Kritas, S. K., Kyriakis, C. S., Tzika, E. and Kyriakis, S. C. (2005). Sow performance in an endemically porcine reproductive and respiratory syndrome (PRRS)-infected farm after sow vaccination with an attenuated PRRS vaccine. *Veterinary Microbiology* **111**, 151-157.

Amano, H., Shibata, M., Kajio, N., Morozumi, T. (1994). Pathologic observations of pigs intranasally inoculated with serovar 1, 4 and 5 of *Haemophilus parasuis* using immunoperoxidase method. *Journal of Veterinary Medical Science* **56**, 639-644

Anderson, R. M. and May, R.M. (1992). In *Infectious diseases of humans: dynamics and* control, pp. 122-143. New York, USA: Oxford University Press Inc.

Andreyev, V. G., Wesley, R. D., Mengeling, W. L., Vorwald, A. C. and Lager, K. M. (1997). Genetic variation and phylogenetic relationships of 22 porcine reproductive and respiratory syndrome virus (PRRSV) field strains based on sequence analysis of open reading frame 5. *Archives of Virology* **142**, 993-1001.

Bartlett, M. S. (1957). Measles Periodicity and Community Size. *Journal of the Royal Statistical Society Series A (General)* **120**, 48-70.

Batista, L., Pijoan, C., Dee, S., Olin, M., Molitor, T., Joo, H. S., Xiao, Z. and Murtaugh, M. (2004). Virological and immunological responses to porcine reproductive and respiratory syndrome virus in a large population of gilts. *Canadian Journal of Veterinary Research* **68**, 267-273.

Bautista, E. M. and Molitor, T. W. (1997). Cell-mediated immunity to porcine reproductive and respiratory syndrome virus in swine. *Viral Immunology* **10**, 83-94.

Baysinger, A. K., Dewey, C. E., Straw, B. E., Brumm, M. C., Schmitz, J., Doster, A. and Kelling, C. (1997). Risk factors associated with endemic reproductive deficiencies caused by PRRSV infection. *Journal of Swine Health and Production* **5**, 179-187.

Black, F. L. (1966). Measles endemicity in insular populations: Critical community size and its evolutionary implication. *Journal of Theoretical Biology* **11**, 207 - 211.

Bloemraad, M., De Kluijver, E. P., Petersen, A., Burkhardt, G. E. and Wensvoort, G. (1994). Porcine reproductive and respiratory syndrome: Temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. *Veterinary Microbiology* **42**, 361-371.

Bossé, J. T., Janson, H., Sheehan, B. J., Beddek, A. J., Rycroft, A. N., Kroll, J. S., Langford, P. R. (2002). *Actinobacillus pleuropneumoniae*: pathobiology and pathogenesis of infection. *Microbes and Infection* **4**, 225-235

Bötner, A., Nielsen, J. and Bille-Hansen, V. (1994). Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. *Veterinary Microbiology* **40**, 351-360.

Bötner, A., Strandbygaard, B., Sorensen, K. J., Have, P., Madsen, K. G., Madsen, E. S. and Alexandersen, S. (1997). Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. *The Veterinary record* **141**, 497-499.

Brockmeier, S. L. and Lager, K. M. (2002). Experimental airborne transmission of porcine reproductive and respiratory syndrome virus and *Bordetella bronchiseptica*. *Veterinary Microbiology* **89**, 267-275.

Burch, D. G. S. (1982). The incidence and distribution of lung lesions, associated with enzootic pneumonia, in pigs from 2 farms, and the effect of the extent of these lesions on weight gains. *Proceedings of the 7th International Pig Veterinary Society Congress*, **95.**

Burch, D. G. S (2005). Problems of antibiotic resistance in pigs in the UK. *In Practice* **27**, 37-43.

Carman, S., Sanford, S. E. and Dea, S. (1995). Assessment of seropositivity to porcine reproductive and respiratory syndrome (PRRS) virus in Ontario – 1978 to 1982. *Canadian Veterinary Journal-Revue Veterinaire Canadienne* **36**, 776-777.

Chang, C. C., Yoon, K. J., Zimmerman, J. J., Harmon, K. M., Dixon, P. M., Dvorak, C. M. T. and Murtaugh, M. P. (2002). Evolution of porcine reproductive and respiratory syndrome virus during sequential passages in pigs. *Journal of Virology* **76**, 4750-4763.

Christensen, G. and Mousing, J. (1992). Respiratory system. In *Diseases of Swine* (7th Edition), pp. 128 – 162. Ames, Iowa, Iowa state, USA: University Press.

Christensen, N. H. (1995). Evaluation of the effects of enzootic pneumonia in pigs on weight gain and days to slaughter under New Zealand conditions. *New Zealand Veterinary Journal* **43**, 146 - 148.

Christianson, W. T., Collins, J. E., Benfield, D. A., Harris, L., Gorcyca, D. E., Chladek, D. W., Morrison, R. B. and Joo, H. S. (1992). Experimental reproduction of swine infertility and respiratory syndrome in pregnant sows. *American Journal of Veterinary Research* **53**, 485-488.

Christianson, W. T., Choi, C. S., Collins, J. E., Molitor, T. W., Morrison, R. B. and Joo, H. S. (1993). Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. *Canadian Journal of Veterinary Research* **57**, 262-268.

Christianson, W. T. and Joo, H. (1994). Porcine reproductive and respiratory syndrome: A review. *Swine Health and Production* **2**, 10-28.

Christopher-Hennings, J., Nelson, E. A., Nelson, J. K., Hines, R. J., Swenson, S. L., Hill, H. T., Zimmerman, J. J., Katz, J. B., Yaeger, M. J. and Chase, C. C. L. (1995). Detection of porcine reproductive and respiratory syndrome virus in boar semen by PCR. *Journal of Clinical Microbiology* **33**, 1730-1734.

Cockburn, A. (1963). In *The Evolution and Eradication of Infectious Diseases (Baltimore, John Hopkins Press).*

Dee, S. A., Morrison, R. B. and Joo, H. S. (1993). Eradicating porcine reproductive and respiratory syndrome (PRRS) virus using two-site production and nursery depopulation. *Swine Health and Production* **1**, 20-23.

Dee, S. A. and Joo, H. S. (1994a). Prevention of the spread of porcine reproductive and respiratory syndrome virus in endemically infected pig herds by nursery depopulation. *The Veterinary Record* **135**, 6-9.

Dee, S. A. and Joo, H. S. (1994b). Recurrent reproductive failure associated with porcine reproductive and respiratory syndrome in a swine herd. *Journal of the American Veterinary Medical Association* **205**, 1017-1018.

Dee, S. A., Joo, H.S., Pijoan, C. (1994c). Controlling the spread of PRRS virus in the breeding herd through management of the gilt pool. *Swine Health and Production* **3**, 64-69.

Dee, S. A., Joo, H. S. and Polson, D. D. (1996). Improved performance of a large pig complex after sequential nursery depopulation. *The Veterinary Record* **138**, 31-34.

Dee, S. A., Joo, H. S., Park, B. K., Pijoan, C., Molitor, T. W., Collins, J. E., King, V. and Poison, D. D. (1997). Evaluation of the effects of nursery depopulation on the persistence of porcine reproductive and respiratory syndrome virus and the productivity of 34 farms. *The Veterinary Record* **140**, 247-248.

Dee, S. A., Joo, H. S., Park, B. K., Molitor, T. W. and Bruna, G. (1998a). Attempted elimination of porcine reproductive and respiratory syndrome virus from a seedstock farm by vaccination of the breeding herd and nursery depopulation. *The Veterinary Record* **142**, 569-572.

Dee, S. A. and Molitor, T. W. (1998b). Elimination of porcine reproductive and respiratory syndrome virus using a test and removal process. *The Veterinary Record* **143**, 474-476.

Dee, S. A., Molitor, T. W. and Rossow, K. D. (2000). Epidemiological and diagnostic observations following the elimination of porcine reproductive and respiratory syndrome virus from a breeding herd of pigs by the test and removal protocol. *The Veterinary Record* **146**, 211-213.

Dee, S. A., Bierk, M.D., Deen, J., Molitor, T.W (2001). An evaluation of test and removal for the elimination of porcine reproductive and respiratory syndrome virus from 5 swine farms. *Canadian Journal of Veterinary Research* **65**, 22-27.

Desrosiers, R. and Boutin, M. (2002). An attempt to eradicate porcine reproductive and respiratory syndrome virus (PRRSV) after an outbreak in a breeding herd: Eradication strategy and persistence of antibody titers in sows. *Journal of Swine Health and Production* **10**, 23-25.

Dohoo, I., Martin, W. and Stryhn, H. (2003). Model-building strategies. In *Veterinary Epidemiologic Research*, 1st edn, pp. 317-332. Edited by S Margaret McPike. AVC Inc: Charlottetown, Prince Edward Island, Canada.

Drew, T. W. (2000). A review of evidence for immunosuppression due to Porcine Reproductive and Respiratory Syndrome Virus. *Veterinary Research* **31**, 27-39.

Easterday, B. C. and Van Reeth, K. (1992). Swine Influenza. In *Diseases of Swine* (7th Edition), pp. 277–290. Ames, Iowa, Iowa state, USA: University Press.

Edwards, S., Robertson, J., Wilesmith, J., Ryan, J., Kilner, C., Paton, D. J., Drew, T. W., Brown, I. and Sands, J. (1992). PRRS ("Blue-Eared Pig Disease) in Great Britain. *AASP Internaltional PRRS Symposium Edition*, 32 - 36.

Evans, C. M., Medley, G. F. and Green, L.E. (2008). Porcine reproductive and respiratory syndrome virus (PRRSV) in GB pig herds: farm characteristics associated with heterogeneity in seroprevalence. *BMC Veterinary Research* **4**, 48.

Evans, C. M., Medley, Creasey, S. J., G. F., Green., L. E. (2009). A stochastic mathematical model of the within-herd transmission dynamics of porcine reproductive and respiratory syndrome virus (PRRSV): fade-out and persistence. *Preventive Veterinary Medicine.* **93**, 248 - 257

Fano, E., Olea, L. and Pijoan, C. (2005). Eradication of porcine reproductive and respiratory syndrome virus by serum inoculation of naive gilts. *Canadian Journal of Veterinary Research-Revue Canadienne De Recherche Veterinaire* **69**, 71-74.

Ferguson, N. M., Donnelly, C. A. and Anderson, R. M. (1999). Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **354**, 757-768.

Freese, W. R. and Joo, H. S. (1994). Cessation of porcine reproductive and respiratory syndrome (PRRS) virus spread in a commercial swine herd. *Swine Health and Production* **2**, 13-15.

Gay, N. J. (1996). Analysis of serological surveys using mixture models: Application to a survey of parvovirus B19. *Statistics in Medicine* **15**, 1567-1573.

Goldberg, T. L., Weigel, R. M., Hahn, E. C. and Scherba, G. (2000). Associations between genetics, farm characteristics and clinical disease in field outbreaks of porcine reproductive and respiratory syndrome virus. *Preventive Veterinary Medicine* **43**, 293-302.

Goodwin, R. F. W. (1971). Economics of enzootic pneumonia. *The Veterinary Record* **89**, 77-&.

Gordon, S. C. (1992). Effects of blue-eared pig-disease on a breeding and fattening unit. *The Veterinary Record* **130**, 513-514.

Hanada, K., Suzuki, Y., Nakane, T., Hirose, O. and Gojobori, T. (2005). The Origin and Evolution of Porcine Reproductive and Respiratory Syndrome Viruses. *Molecular Biology and Evolution* **22**, 1024-1031.

Harms, P. A., Sorden, S. D., Halbur, P. G., Bolin, S. R., Lager, K. M., Morozov, I. and Paul, P. S. (2001). Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. *Veterinary Pathology* **38**, 528-539.

Hill, H. (1990). Overview and history of mystery swine disease (Swine Infertility Reproductory Syndrome). *Proceedings of the mystery swine disease committee meeting, Denver, CO, 6 Oct 1990.*

Hopper, S. A., White, M. E. and Twiddy, N. (1992). An outbreak of blue-eared pig disease (porcine reproductive and respiratory syndrome) in four pig herds in Great Britain. *The Veterinary Record.* **131**, 140-144.

Houben, S., van Reeth, K. and Pensaert, M. B. (1995). Pattern of infection with the porcine reproductive and respiratory syndrome virus on swine farms in Belgium. *Zentralblatt fur Veterinarmedizin Reihe B Journal of veterinary medicine Series B* **42**, 209-215.

Huhn, R. G. (1970). Swine enzootic pneumonia – incidence and effect on rate of body weight gain. *American Journal of Veterinary Research* **31**, 1097.

Johnson, W., Roof, M., Vaughn, E., Christopher-Hennings, J., Johnson, C. R. and Murtaugh, M. P. (2004). Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. *Veterinary Immunology and Immunopathology* **102**, 233-247.

Joo, H. S., Park, B. K., Dee, S. A. and Pijoan, C. (1997). Indirect fluorescent igm antibody response of pigs infected with porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* **55**, 303-307.

Keeling, M. J. and Grenfell, B. T. (1997). Disease Extinction and Community Size: Modeling the Persistence of Measles. *Science* **275**, 65-67.

Keeling, M. J. (2005). Models of foot-and-mouth disease. *Proceedings of the Royal Society B-Biological Sciences* **272**, 1195-1202.

KilBride, A. L., Gillman, C. E. and Green, L. E. (2009). A cross-sectional study of the prevalence of lameness in finishing pigs, gilts and pregnant sows and associations with limb lesions and floor types on commercial farms in England. *Animal Welfare* **18**, 215-224.

Kranker, S., Nielsen, J., Bille-Hansen, V. and Bötner, A. (1998). Experimental inoculation of swine at various stages of gestation with a Danish isolate of porcine reproductive and respiratory syndrome virus (PRRSV). *Veterinary Microbiology* **61**, 21 - 31.

Kristensen, C. S., Bötner, A., Takai, H., Nielsen, J. P. and Jorsal, S. E (2004). Experimental airborne transmission of PRRS virus. *Veterinary Microbiology* **99**, 197 - 202.

Labarque, G. G., Nauwynck, H. J., Van Reeth, K. and Pensaert, M. B. (2000). Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. *Journal of General Virology* **81**, 1327-1334.

Labarque, G., Reeth, K. V., Nauwynck, H., Drexler, C., Gucht, S. V. and Pensaert, M. (2004). Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy. *Vaccine* **22**, 4183-4190.

Lindhaus, W. and Lindhaus, B. (1991). Mystery swine disease. *Praktische Tierarzt* **72**, 423-425.

Löpez Fuertes, L., Domenech, N., Alvarez, B., Ezquerra, A., Dominguez, J., Castro, J. M. and Alonso, F. (1999). Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. *Virus Research* **64**, 33-42.

Losinger, W. C., Bush, E. J., Smith, M. A. and Corso, B. A. (1998). Mortality attributed to respiratory problems among finisher pigs in the United States. *Preventive Veterinary Medicine* **37**, 21-31.

Lurette, A., Belloc, C., Touzeau, S., Hoch, T., Seegers, H. and Fourichon, C. (2008). Modelling batch farrowing management within a farrow-to-finish pig herd: influence of management on contact structure and pig delivery to the slaughterhouse. *Animal* **2**, 105-116

MacKenzie, K. and Bishop, S. C. (2001). Developing stochastic epidemiological models to quantify the dynamics of infectious diseases in domestic livestock. *Journal of Animal Science* **79**, 2047-2056.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Virjens, B. and de Kruif, A. (1999). Risk indicators for the seroprevalence of *Mycoplasma hyopneumoniae*, porcine influenza viruses and Aujeszky's disease virus in slaughter pigs from fattening pig herds. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health* **46**, 341-352.

Maes, D., Deluyker, H., Verdonck, M., Castryck, F., Miry, C., Vrijens, B. and de Kruif, A. (2000). Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. *Veterinary Research* **31**, 313-327.

Marois, C., Gottschalk, M., Morvan, H., Fablet, C., Madec, F., Kobisch, M. (2008). Experimental infection of SPF pigs with Actinobacillus pleuropneumoniae serotype 9 alone or in association with *Mycoplasma hyopneumoniae*. *Veterinary Microbiology* **135**, 283-291

Meng, X. J., Paul, P. S., Halbur, P. G. and Lum, M. A. (1995). Phylogenetic analyses of the putative M(ORF-6)-gene and N(ORF-7)-gene of porcine reproductive and respiratory syndrome virus (PRRSV) – implications for the existence of 2 genotypes of PRRSV in the USA and Europe. *Archives of Virology* **140**, 745-755.

Mengeling, W. L., Lager, K. M. and Vorwald, A. C. (1994). Temporal characterisation of transplacental infection of porcine foetuses with porcine reproductive and respiratory syndrome virus. *American Journal of Veterinary Research* **55**, 1391-1398.

Mengeling, W. L., Vorwald, A. C., Lager, K. M. and Brockmeier, S. L. (1996). Comparison among strains of porcine reproductive and respiratory syndrome virus for their ability to cause reproductive failure. *American Journal of Veterinary Research* **57**, 834-839.

Mengeling, W. L., Lager, K. M., Vorwald, A. C. and Clouser, D. F. (2003a). Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome. *Veterinary Microbiology* **93**, 25-38.

Mengeling, W. L., Lager, K. M., Vorwald, A. C. and Koehler, K. J. (2003b). Strain specificity of the immune response of pigs following vaccination with various strains of porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* **93**, 13-24.

Meulenberg, J. J. M., Hulst, M. M., De Meijer, E. J., Moonen, P. L. J. M., Den Besten, A., De Kluyver, E. P., Wensvoort, G. and Moormann, R. J. M.

(1993). Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* **192**, 62-72.

Meulenberg, J. J. M., Petersen-Den Besten, A., De Kluyver, E. P., Moormann, R. J. M., Schaaper, W. M. M. and Wensvoort, G. (1995). Characterization of proteins encoded by ORFs 2 to 7 of Lelystad virus. *Virology* **206**, 155-163.

Mortensen, S., Stryhn, H., Sogaard, R., Boklund, A., Stärk, K. D., Christensen, J. and Willeberg, P. (2002). Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus. *Preventive Veterinary Medicine* **53**, 83 - 101.

Mousing, J., Permin, A., Mortensen, S., Bötner, A. and Willeberg, P. (1997). A case-control questionnaire survey of risk factors for porcine reproductive and respiratory syndrome (PRRS) seropositivity in Danish swine herds. *Veterinary Microbiology* **55**, 323-328.

Neumann, E. J., Kliebenstein, J. B., Johnson, C. D., Mabry, J. W., Bush, E. J., Seitzinger, A. H., Green, A. L. and Zimmerman, J. J. (2005). Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *Journal of the American Veterinary Medical Association* **227**, 385-392.

Nodelijk, G., Wensvoort, G., Kroese, B., Leengoed, L. v., Colijn, E. and Verheijden, J. (1996). Comparison of a commercial ELISA and an immunoperoxidase monolayer assay to detect antibodies directed against porcine respiratory and reproductive syndrome virus. *Veterinary Microbiology* **49**, 285-295.

Nodelijk, G., Van Leengoed, L. A. M. G., Schoevers, E. J., Kroese, A. H., De Jong, M. C. M., Wensvoort, G. and Verheijden, J. H. M. (1997). Seroprevalence of porcine reproductive and respiratory syndrome virus in Dutch weaning pigs. *Veterinary Microbiology* **56**, 21-32.

Nodelijk, G., De Jong, M. C. M., Van Nes, A., Vernooy, J. C. M., Van Leengoed, L. A. M. G., Pol, J. M. A. and Verheijden, J. H. M. (2000). Introduction, persistence and fade-out of porcine reproductive and respiratory syndrome virus in a Dutch breeding herd: A mathematical analysis. *Epidemiology and Infection* **124**, 173-182.

O'Connor, M., Fallon, M. and O'Reilly, P. J. (2002). Detection of antibody to porcine reproductive and respiratory syndrome (PRRS) virus: reduction of cut-off value of an ELISA, with confirmation by immunoperoxidase monolayer assay. *Irish Veterinary Journal* **55**, 73 - 75.

Opriessnig, T., Thacker, E. L., Yu, S., Fenaux, M., Meng, X. J. and Halbur, P. G. (2004). Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneamoniae* and porcine circovirus type 2. *Veterinary Pathology* **41**, 624-640.

Otake, S., Dee, S. A., Jacobson, L., Pijoan, C. and Torremorell, M. (2002a). Evaluation of aerosol transmission of porcine reproductive and respiratory syndrome virus under controlled field conditions. *The Veterinary Record* **150**, 804-808.

Otake, S., Dee, S. A., Rossow, K. D., Deen, J., Joo, H. S., Molitor, T. W. and Pijoan, C. (2002b). Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *Journal of Swine Health and Production* **10**, 59-65.

Otake, S., Dee, S. A., Moon, R. D., Rossow, K. D., Trincado, C. and Pijoan, C. (2004). Studies on the carriage and transmission of porcine reproductive and respiratory syndrome virus by individual houseflies (*Musca domestica*). *The Veterinary record* **154**, 80-85.

Papatsiros, V. G., Alexopoulos, C., Kritas, S. K., Koptopoulos, G., Nauwynck, H. J., Pensaert, M. B. and Kyriakis, S. C. (2006). Long-term administration of a commercial porcine reproductive and respiratory syndrome virus (PRRSV)-inactivated vaccine in PRRSV-endemically infected sows. *Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health* **53**, 266-272.

Paton, D. J., Brown, I. H., Scott, A. C., Done, S. H. and Edwards, S. (1992). Isolation of a Lelystad virus-like agent from British pigs and scanning electron microscopy of infected macrophages. *Veterinary Microbiology* **33**, 195-201.

Peet, R. L., Fry, J., Lloyd, J., Henderson, J., Curran, J., Moir, D. (1983). *Haemophilus parasuis* septicemia in pigs. *Australian Journal of Veterinary Research.* **60**, 187.

Pejsak, Z. and Markowska-Daniel, I. (1997). Losses due to porcine reproductive and respiratory syndrome in a large swine farm. *Comparative Immunology, Microbiology and Infectious Diseases* **20**, 345-352.

Pesch, S., Meyer, C. and Ohlinger, V. F. (2005). New insights into the genetic diversity of European porcine reproductive and respiratory syndrome virus (PRRSV). *Veterinary Microbiology* **107**, 31-48.

Pitkin, A., Deen, J. and Dee, S. (2009). Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* **136**, 1-7.

Plana, J., Vayreda, M., Vilarrasa, J., Bastons, M., Rosell, R., Martinez, M., San Gabriel, A., Pujols, J., Badiola, J. L. and Ramos, J. A. (1992). Porcine epidemic abortion and respiratory syndrome (mystery swine disease). Isolation in Spain of the causative agent and experimental reproduction of the disease. *Veterinary Microbiology* **33**, 203-211.

Plana-Duran, J., Bastons, M., Urniza, A., Vayreda, M., Vilax X. and Mane, H. (1997). Efficacy of an inactivated vaccine for prevention of reproductive failure induced by porcine reproductive and respiratory syndrome virus. *Veterinary Microbiology* **55**, 361-370.

Plomgaard, J. (1998). Eradication of PRRS from the swine herd. *Proceedings AD Leman Swine Conference*, 194.

Pol, J. M. A., van Leengoed, L. A. M. G., Stockhofe, N., Kok, G. and Wensvoort, G. (1997). Dual infections of PRRSV/influenza or PRRSV/*Actinobacillus pleuropneumoniae* in the respiratory tract. *Veterinary Microbiology* **55**, 259-264.

Potter, R. A. (1994). Non-transmission of porcine reproductive and respiratory syndrome virus by seropositive pigs from an infected herd. *The Veterinary Record* **134**, 304 - 305.

Prieto, C., Sanchez, R., Martin-Rillo, S., Suarez, P., Simarro, I., Solana, A. and Castro, J. M. (1996). Exposure of gilts in early gestation to porcine reproductive and respiratory syndrome virus. *The Veterinary Record* **138**, 536-539.

Prieto, C., Suárez, P., Simarro, I., García, C., Martín-Rillo, S. and Castro, J. M. (1997). Insemination of susceptible and preimmunized gilts with boar semen containing porcine reproductive and respiratory syndrome virus. *Theriogenology* **47**, 647-654.

Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I. and Lewis T. (2000). *A Users Guide to MLwiN*, Version 2.1. Multilevel Models Project, Institute of Education, University of London, UK.

Regula, G., Lichtensteiger, C. A., Mateus-Pinilla, N. E., Scherba, G., Miller, G. Y. and Weigel, R. M. (2000). Comparison of serologic testing and slaughter evaluation for assessing the effects of subclinical infection on growth of pigs. *Journal of the American Veterinary Medical Association* **217**, 888 - 895.

Robertson, I. B. (1992). Transmission of blue-eared pig-disease. *The Veterinary Record* **130**, 478-479.

Ross, R. F. (1992). Mycoplasmal diseases. In *Diseases of Swine* (7th Edition), pp. 495–501. Ames, Iowa, Iowa state, USA: University Press.

Rosell, C., Segalés, J., Plana-Durán, J., Balasch, M., Rodríguez-Arrioja, G. M., Kennedy, S., Allan, G. M., McNeilly, F., Latimer, K. S. and Domingo, M. (1999). Pathological, Immunohistochemical, and In-situ Hybridization Studies of Natural Cases of Postweaning Multisystemic Wasting Syndrome (PMWS) in Pigs. *Journal of Comparative Pathology* **120**, 59-78.

Rovira, A., Balasch, M., Segales, J., Garcia, L., Plana-Duran, J., Rosell, C., Ellerbrok, H., Mankertz, A. and Domingo, M. (2002). Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. *Journal of Virology* **76**, 3232-3239.

Scortti, M., Prieto, C., Martinez-Lobo, F. J., Simarro, I. and Castro, J. M. (2006). Effects of two commercial European modified-live vaccines against porcine reproductive and respiratory syndrome viruses in pregnant gilts. *The Veterinary Journal* **172**, 506-514.

Shirai, J., Kanno, T., Tsuchiya, Y., Mitsubayashi, S. and Seki, R. (2000). Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses. *Journal of Veterinary Medical Science* **62**, 85-92.

Stärk, K. D. C., Nicolet, J. and Frey, J. (1998). Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. *Applied and Environmental Microbiology* **64**, 543-548.

Stärk, K. D. C. (2000). Epidemiological Investigation of the Influence of Environmental Risk Factors on Respiratory Diseases in Swine--A Literature Review. *The Veterinary Journal* **159**, 37-56.

Stevenson, G. W., Van Alstine, W. G., Kanitz, C. L. and Keffaber, K. K. (1993). Endemic porcine reproductive and respiratory syndrome virus infection of nursery pigs in two swine herds without current reproductive failure. *Journal of Veterinary Diagnostic Investigation* **5**, 432-434.

Straw, B. E., Neubauer, G. D. and Leman, A. D. (1983). Factors affecting mortality in finishing pigs. *Journal of the American Veterinary Medical Association* **183**, 452-455.

Straw, B. E., Tuovinen, V. K. and Bigras-Poulin, M. (1989). Estimation of the cost of pneumonia in swine herds. *Journal of the American Veterinary Medical Association* **195**, 1702-1706.

Straw, B. E. S., S. J; Yeager, A. E (1990). Effect of pneumonia on growth rate and feed efficiency of minimal disease pigs exposed to *Actinobacillus*

pleuropneumoniae and *Mycoplasma hyopneumoniae*. *Preventive Veterinary Medicine* **9**, 287 - 294.

Taylor, N. (2003). *Review of the use of models in informing disease control policy development and adjustment*, pp. 1-94. VEERU, School of Agriculture, Policy and Development, Earley Gate, Reading, UK.

Terpstra, C., Wensvoort, G., Van Leengoed, LAMG. (1992). Persistence of Lelystad virus in herds affected by porcine epidemic abortion and respiratory syndrome. *Proceedings of 12th International Pig Veterinary Society Congress, The Hague, The Netherlands*, 118.

Thacker, E. L., Halbur, P. G., Ross, R. F., Thanawongnuwech, R. and Thacker, B. J. (1999). *Mycoplasma hyopneumoniae* potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. *Journal of Clinical Microbiology* **37**, 620-627.

Thacker, E. L. (2001). Immunology of the porcine respiratory disease complex. *Immunology* **17**, 551 - 565.

Thacker, E. L., Thacker, B. J. and Janke, B. H. (2001). Interaction between *Mycoplasma hyopneumoniae* and swine influenza virus. *Journal of Clinical Microbiology* **39**, 2525-2530.

Trincado, C., Dee, S., Jacobson, L., Otake, S., Rossow, K. and Pijoan, C. (2004). Attempts to transmit porcine reproductive and respiratory syndrome virus by aerosols under controlled field conditions. *The Veterinary Record* **154**, 294-297.

Turner, M. J., Medley, G. F., Woodbine, K. A., Slevin, J. A. and Green, L. E. (2009). The relationship between porcine circovirus 2 antigen score and antibody titre and histology of lymph nodes in 375 euthanased sick and healthy pigs from 113 British pig farms with and without postweaning multisystemic wasting syndrome. *Preventive Veterinary Medicine* **88**, 213-219.

Van Alstine, W. G., Stevenson, G. W. and Kanitz, C. L. (1996). Porcine reproductive and respiratory syndrome virus does not exacerbate *Mycoplasma hyopneumoniae* infection in young pigs. *Veterinary Microbiology* **49**, 297-303.

Van Reeth, K., Nauwynck, H. and Pensaert, M. (1996). Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study. *Veterinary Microbiology* **48**, 325-335.

Wensvoort, G., Terpstra, C., Pol, J., ter Laak, E., Bloemraad, M., De Kluijver, E.P., Kragten, E., van Buiten, L., Den Besten, A., Wagenaar, F.,

Broekhuijsen, J., Moonen, P.L.J.M., Zetstra, T., de Boer, E., Tibben, H., De Jong, M. F., P., v. t. V., G.J.R., G., van Gennep, J., Voets M. T. H., Verheijden, J. H. M. and Braamskamp, J. (1991). Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Veterinary Quarterly* **13**, 121-130.

Wensvoort, G., de Kluyver, E. P., Pol, J. M. A., Wagenaar, F., Moormann, R. J. M., Hulst, M. M., Bloemraad, R., Besten, A. d., Zetstra, T. and Terpstra, C. (1992). Lelystad virus, the cause of porcine epidemic abortion and respiratory syndrome: a review of mystery swine disease research at Lelystad. *Veterinary Microbiology* **33**, 185-193.

Whittemore, C. T. (1993). In *The science and practice of pig production (2nd edition).* Harlow, Essex. Longman Scientific and Technical.

Wills, R. W., Zimmerman, J. J., Swenson, S. L., Yoon, K. J., Hill, H. T., Bundy, D. S. and McGinley, M. J. (1997a). Transmission of PRRSV by direct, close, or indirect contact. *Swine Health and Production* **5**, 213-218.

Wills, R. W., Zimmerman, J. J., Yoon, K. J., Swenson, S. L., Huffman, L. J., McGinley, M. J., Hill, H. T. and Platt, K. B. (1997b). Porcine reproductive and respiratory syndrome virus: Routes of excretion. *Veterinary Microbiology* **57**, 69-81.

Wills, R. W., Doster, A. R., Galeota, J. A., Sur, J. H. and Osorio, F. A. (2003). Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. *Journal of Clinical Microbiology* **41**, 58-62.

Woodbine, K. A., Medley, G. F., Slevin, J., Kilbride, A. L., Novell, E. J., Turner, M. J., Keeling, M. J. and Green, L. E. (2007). Spatiotemporal patterns and risks of herd breakdowns in pigs with postweaning multisystemic wasting syndrome. *The Veterinary Record* **160**, 751-762.

Yang, J. S., Moon, H. J., Lee, C. S., Park, S. J., Song, D. S., Kang, B. K., Choi, J. U. and Park, B. K. (2008). Elimination of porcine reproductive and respiratory syndrome virus from a seedstock breeding farm and a supplying boar stud by a modified test and removal method. *The Veterinary Record* **162**, 333-337.

Yoon, K. J., Zimmerman, J. J., Swenson, S. L., McGinley, M. J., Eernisse, K. A., Brevik, A., Rhinehart, L. L., Frey, M. L., Hill, H. T. and Platt, K. B. (1995). Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection. *Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc* **7**, 305 - 312.

Yorke, J. A., Nathanson, N., Pianigiani, G. and Martin, J. (1979). Seasonality and the requirements for perpetuation and eradication of viruses in populations. *American Journal of Epidemiology* **109**, 103 - 123.

Zimmerman, D. R., Spear, M. L. and Switzer, W. P. (1973). Effect of *Mycoplasma Hyopneumoniae* Infection, Pyrantel Treatment and Protein Nutrition on Performance of Pigs Exposed to Soil Containing *Ascaris Suum* Ova. *Journal of Animal Science* **36**, 894-897.

Zimmerman, J. J., Yoon, K. J., Pirtle, E. C., Wills, R. W., Sanderson, T. J. and McGinley, M. J. (1997). Studies of porcine reproductive and respiratory syndrome (PRRS) virus infection in avian species. *Veterinary Microbiology* **55**, 329 - 336.

Zimmerman, J. J. (2003). Historical overview: In 2003 PRRS Compendium: second edition, pp. 3. Iowa State University, Ames, Iowa.

Appendix 1